1952

A nitrification procedure for predicting the availability of nitrogen to corn on Iowa soils

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A NITRIFICATION PROCEDURE FOR PREDICTING THE AVAILABILITY OF NITROGEN TO CORN ON IOWA SOILS

by

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A Dissertation Submitted to the
Graduate Faculty in Partial Fulfillment of
The Requirements for the Degree of
DOCTOR OF PHILOSOPHY

Major Subject: Soil Fertility

Approved:

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Iowa State College
1952

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INTRODUCTION

The use of green manure crops and animal manure as a means of increasing plant growth is an ancient practice as indicated by records of early Greek and Roman writings and records of ancient Chinese civilization. This presents strong evidence that nitrogen has been deficient for cultivated crops since the beginning of agriculture. Chemical nitrogen salts, however, were not generally used until during the last 100 years (22, 27).

As early as 1660, Sir Kenelin Digby mentioned the value of nitrates in agriculture (22, 88). He attributed the growth of plants to "nutritional and attractional" powers of a "nitrous salt" after he had increased crop yields by the application of saltpeter. About 200 years passed before nitrogen was shown to be an essential element for plant growth by Lawes, Gilbert and Pugh of the Rothamsted Experiment Station (22). In 1856, Boussingault and Ville independently published experimental results which proved nitrates are markedly beneficial to plant growth.

Nothing was known about the process of nitrification during the 17th and 18th centuries but methods had been evolved for the preparation of nitre beds as a source of material for gunpowder. In the early 1800's, it was shown that nitrate can be formed by electrical discharge and it was believed that nitrate in the soil was formed in this manner. Pasteur, in 1862, was the first to suggest that nitrate formation might be a bacteriological process but it remained for Schloesing and Muntz, two Frenchmen (102), in 1877 to provide experimental evidence to prove this was true.

Since 1900 there has become an increasing awareness of the importance of nitrogen in crop production. Parker (90) has pointed out that nitrogen is the most commonly deficient nutrient in the arable soils of the world. Within the last 25 years, a world nitrogen industry has developed and world consumption of chemical fertilizers has increased manyfold.

For many years it has been the goal of agricultural scientists to find a means by which it will be possible to predict with a fair degree of accuracy the response that might be expected from different fertilizer practices on crops under various conditions. A laboratory test for use in a soil testing laboratory for predicting response to fertilizer application should be simple to perform, require a minimum of time to complete, be accurate, and inexpensive. Moreover, the laboratory results should be correlated with field and greenhouse experiments covering a wide range of soil and crop conditions.

Fairly good success has been obtained in developing phosphorus, potassium, and lime requirement tests and they are used generally in many laboratories. The problem of predicting nitrogen response is different from predicting response from other elements, since nitrogen occurs largely in the organic fraction of the soil. It occurs either as stabilized humus or the still undecomposed particles of vegetable or animal origin. Only a small quantity of inorganic nitrogen occurs at any one time in the soil. The amount and kind of recently added organic matter, temperature, moisture, and aeration are some of the more important factors influencing the quantity of inorganic nitrogen present in the soil at any given time. Nitrate and ammonia are the principal forms of inorganic nitrogen found in soils. Nitrogen can be utilized by plants as either nitrate or ammonia (55). Nitrate is formed from ammonia by bacteriological oxidation and it may be considered the end product of organic matter decomposition. Hiltbold, Bartholomew and Werkman (52) pointed out that, under soil conditions favorable for microbiological activity, the depletion and replenishment of inorganic nitrogen occurs continuously. With the aid of heavy nitrogen as a tracer, they found for every two parts of nitrogen ammonified on a fallow soil, one part was reutilized by soil microflora.

The amount of organic matter in a soil is influenced by many factors, including type of plant or animal residue and climatic conditions. Jenny (56) has shown that the organic matter content of soil from 0-7 inches deep in a line extending from Canada southward to the Gulf of Mexico decreases two or three times for each 10^{o} increase in mean annual temperature. In the south, it is practically impossible to maintain the organic matter at a high level because the high mean annual temperature militates against nitrogen accumulation by favoring decomposition.

The organic matter content of the soil has been found to be a good measure of soil productivity (63, 103, 117). Woodruff (122) has been able to evaluate the average annual delivery of nitrogen from the plots on the Sanborn field in Missouri as a function of the amount of nitrogen in the soil and of the kind of crop. Thompson (111) has shown that both nitrogen and organic phosphorus become more resistant to mineralization as the degree of soil organic matter decomposition advances due to cultivation.

Gracie and Khalil (44) have determined "available" nitrogen by hydrolysis with dilute sulphuric acid in an autoclave under 1 atmosphere pressure. They found a correlation of 0.618 between hydrolyzable nitrogen and total nitrogen. The soluble nitrogen was related to crop yields and nitrogen requirement. Jenny and co-workers (57) determined nitrogen requirement by growing Romaine lettuce in the greenhouse for six to eight weeks. They found a good correlation between greenhouse studies and field results. Both of these methods appear to give good results but for use in a soil testing laboratory where a large number of samples are tested they are too long and tedious.

H. Lees and co-workers (66, 67, 68, 69, 70, 71, 72) have obtained valuable information on nitrification from the soil percolation technique which they have developed. Due to differences in the physical condition of soils such a technique does not appear plausible for use in testing a large number of samples.

Harmsen and Lindenbergh (50) point out that the best procedure for a correct estimate of fertilization requirements is a measure of the activity and rate of mineralization of soil organic nitrogen. For this purpose they proposed a procedure that is divided into two steps, (1) extraction of available nitrogen present by a fast growing crop, and (2) regeneration period to determine the speed at which nitrate accumulates in the soil. This procedure is also too long for use in laboratories testing several thousand samples a year.

Black, Nelson and Pritchett (10, 95) have shown a good correlation between mineralizable nitrogen in soils and the response to the application of nitrogen fertilizers on oats or wheat. Small grains differ from corn in the time of year when nitrogen is needed in largest quantities (120, 90). They are also able to compensate for thin stands more readily than corn by stooling and thereby utilize available nitrogen that may be present.

More acres of cultivated land in Iowa are devoted to the production of corn than to any other crop. Although Nelson (86) and Nelson and Black (87) have shown that a relationship exists between the degree of response of corn to nitrogen and the past cropping sequence, at present there is no reliable quick test upon which to base nitrogen recommendations on samples submitted for testing. In an effort to develop a laboratory test that can be used for predicting response from nitrogen fertilizer applications on corn, studies were undertaken with the following objectives.

1. To study factors effecting nitrification and nitrate analysis and to formulate a procedure that can be used as a laboratory test for predicting nitrogen needs.
2. To determine variability in obtaining soil samples for nitrification studies and the effect of time of year in which samples are taken in relation to cropping practices upon nitrification.

3. To investigate in a large number of farmers' samples the relationship between nitrification rate, the available phosphorus and potassium, and the soil pH.
4. To relate nitrification rates with response to nitrogen fertilizer application to corn in field studies conducted from 1943 through 1950.

FACTORS AFFECTING NITRIFICATION AND NITRATE ANALYSIS

Introduction

Nitrification rate is evaluation by measuring the increase in nitrogen in a soil sample after a given period of time in which the soil is incubated at optimum moisture and temperature. Many studies during the last 50 years have been conducted upon nitrification and factors effecting ammonia release and oxidation. The procedures used varied greatly and conflicting results have been obtained.

There are many factors that influence nitrification rate studies including size of soil sample, types of containers, size of particles, presence of nitrifying organisms, soil structure, temperature, aeration, length of incubation period, and the kind and amount of "active" organic matter present.

Historical

The amount of moisture to add to the soil and maintenance of the desired moisture content during the incubation period has been a major problem in nitrification studies. Samples ranging from 50 grams to several thousand grams

(20, 26, 30, 37, 40, 48, 65, 100) have been used. Most laboratories use 100 grams of soil placed in large mouth vessels such as glass tumblers or pint milk bottles. Gainey and Metzler (40) found little if any effect upon nitrification by varying the quantity of soil from 50 to 100 grams. Cheney (20) conducted incubation studies by placing soil samples in large crocks. Russel, Jones and Bahrt (100) used 1000 gram samples in two quart fruit jars. Fraps and Sterges (30, 32) used 200 gram and 100 gram samples in 300 ml or 150 ml pyrex beakers. Halvorson and Caldwell (48) used 60 grams of soil in 6 ounce jelly glasses. Drouineau and Lefevre (26) used 50 grams of soil and 50 grams of quartz sand. Landrau (65), Kubota, Rhoades and Harris (64), Hanway (49) and McCalla and Russel (82, 83) used 100 grams of soil in pint milk bottles. Moisture was maintained in the soil by all of these workers by the addition of distilled water two or three times a week.

Much of the literature is confusing and apparently conflicting in regard to the effect of moisture on nitrate production. Part of this is due to the manner in which the moisture constants were expressed. Most data indicate nitrification is highest within a certain moisture range and decreases with either more or less moisture. Waksman (114) points out that a sufficient moisture content in sands may not be sufficient for finer textured soils. Nitrifying organisms are very sensitive to drying. Nitrification is at its highest when the soil contains about 55 per cent of its water holding capacity. Excessive moisture is more injurious than insufficient moisture. Greaves and Carter (45) using 100 grams of soil with 2 grams of dried blood in glass tumblers in which distilled water was added every third day, found the highest nitrification was at 60 per cent of water holding capacity. They studied 22 soils in which Hilgard's water holding capacity varied from 31 to 78 per cent. Russel, Jones and Bahrt (100) obtained the highest nitrification at the highest moisture content studied which was one and one-fourth moisture equivalent. Gainey and Metzler (40) report the optimum moisture content for nitrification to be two-thirds of field carrying capacity. Drouineau and Lefevre (26) added 12.5 cc of water to the 100 grams of 1:1 sand and soil mixture. Bhaumik and Clark (9) found the moisture at the aeration porosity limit taken by convention at 50 cm water tension as the point that the general soil microbiological population is provided with the most favorable aeration condition that can exist simultaneously with maximum thickness of capillary moisture film.

Russel, Jones and Bahrt (100) conclude aeration probably is the most uncertain factor in incubation studies. Where soils are tightly compacted or containers sealed, the nitrification rates will be greatly influenced. Gainey and Metzler

(40) found in nitrification studies that as the moisture content of a soil decreases, increasing the compactness from a very loose condition will increase the accumulation of nitrates. In their studies the shape and size of the container as well as methods of preventing evaporation were without effect except when the container was stoppered tightly and the volume of enclosed air was relatively small in proportion to the soil volume. Under poorly aerated or anaerobic conditions, nitrate is assimilated by micro-organisms (24). Fraps and Sterges (33) state that nitrification is decreased by puddling of soils and does not occur to any appreciable extent in water-logged soils. Plummer (92) showed that nitrification took place in sealed containers as long as there was a supply of oxygen and that carbon dioxide had no effect on nitrification as long as oxygen was available. When oxygen became limiting then denitrification resulted. Smith, Brown and Millar (105) added carbon dioxide to the soil as a gas and also as carbonic acid. Treatment with carbonic acid was effective in stimulating nitrate production probably by increasing the solubility of the mineral constituents required by the nitrifying organisms. Carbon dioxide gas did not effect nitrification but the reduction of oxygen too far reduced the nitrification rate. Amer and Bartholomew (4) found the critical oxygen percentage for nitrification in

soil was below 1.0 per cent but greater than 0.4 per cent. About one-half as much nitrate was produced in 2.1 per cent as was produced in 20 per cent oxygen. Reducing oxygen from 20 to 11 per cent had only negligible influence upon nitrification rate.

Microbiological activity in the soil in general has been found to follow Van't Hoff's rule rather closely up to a temperature of 25° C. For each 10° C rise in temperature, the activity increases two or three times, other conditions being constant.(56). Schloesing and Muntz (102) in 1879 were the first to report the influence of temperature upon nitrification. They reported that below a temperature of 5° C nitrification is excessively slow if not completely lacking but becomes appreciable at 12° C. The maximum rate of nitrate production is reached at 37° C and above that there is a rapid diminution. At 50° C only very small amounts of nitrate were obtained and around 55° C there was complete inhibition of nitrification. Russel, Jones and Bahrt (100) obtained greatest nitrification at 35° C with a large reduction at 48° C and 23° C respectively. The optimum temperature for nitrate formation in tropical soil was found to be 35° C by Tandon and Dhar (109). King and Whitson (62) and Panganiban (89) also found 35° C as the optimum temperature for nitrification. Waksman and Madhok (115) found 27° to 37° C to be the optimum range for nitrification with the rate reduced above or below this range. Thompson and Black (111) studied at 30° and 35° C the differences in nitrification between six pairs of virgin and cultivated soils. Two sample sizes in different sized containers with dissimilar moisture contents were used, making it difficult to attribute all of the difference in nitrification to temperature alone. In general nitrification was greater in the soils incubated at 35° C than those incubated at 30° C. Panganiban (89) also found ammonification took place between 15° and 60° C with the greatest rate at higher temperatures. Thompson (110) found the greatest mineralization of nitrogen during a seven day incubation period took place at 60° C to 70° C with a reduction at 50° C and 80° C.

The decomposition of cellulose in soils generally results in immobilization of nitrate nitrogen. Fuller and Jones (35) found the least immobilization of nitrate in the soil during decomposition of cellulose to be at 35° C with the greatest immobilization at 10° to 15° C.

Although Cline (21) recommends that a standard maximum particle size for chemical analysis of soil is needed, not many papers report the screen size used in studying nitrification. Russel, Jones and Bahrt (100), Kubota, Rhoades and Harris (64), Hanway (49), and McCalla and Russel (82, 83) all used soil that had been passed through a one-fourth inch screen prior to air drying. Black, Nelson and Pritchett (10) used soil passed through a 2 mm screen in one study and a 40 mesh sieve in another.

Harmsen and Lindenbergh (50) indicate the initial rapid production of nitrate is the best measure for predicting crop response to nitrogen fertilizer application. Although the quantities of nitrate accumulated at the end of three weeks are not as great as at six or eight weeks, the difference in nitrification rates for samples from different rotation plots at Mandan, North Dakota, are as apparent in the work reported by Allison and Sterling (3). Similar results were obtained by Andharia (5) in studying nitrification of soil samples taken from rotation plots at Clarinda, Iowa, in 1951. Hanway (49) obtained highly significant correlations between two and four week, two and eight week, and four and eight week incubations of samples taken from rotation plots at Lincoln, Nebraska. A high correlation between two and eight week periods was obtained by Landrau (65) also.

Gainey (36) stated that most normal cultivated soils contain active nitrifying organisms. In such soils ammonia does not accumulate beyond a fairly constant equilibrium point. When nitrification is impossible, ammonia accumulates but it soon disappears when conditions for nitrification improve.

In studying causes of low nitrification of certain soils in Texas, Fraps and Sterges (30, 33) found addition of nitrifying soil plus calcium carbonate usually caused nitrification of ammonium sulphate in soils where little or no nitrification took place previously. Addition of inoculating liquid alone produced little or no nitrification. Additions of calcium carbonate or bacteria or both increased nitrification of soil nitrogen in many soils but not in all of them. The increases, if any, were usually relatively small.

In nitrification experiments with ammonium sulphate the number of nitrifying organisms initially present is likely to effect the results. The quantity of nitrates produced increased in general as the quantity of inoculum was increased from 0.1 to 20 grams per 200 grams of sterilized soil. The nitrate production was not linearly related to the amount of inoculum added.

Nitrifying bacteria remain viable for many years in air dried soils. Greaves and Jones (46) found that the nitrifying power of soils was not effected by storage at either 20° C or 30° C for 24 months. Soils stored at either 10° C or 40° C for 24 months had slightly lower nitrifying powers. Fraps and Sterges (30) found that several samples stored in air dried conditions for 14 to 18 years gave the same results when incubated with or without addition of inoculating liquids.

Rao and Dhar (96, 97) propose the theory that nitrification in the soil is not due entirely to bacteria but it is partly due to photochemical reactions taking place at the surface of the soil under the influence of sunlight. They present evidence that sunlight can cause nitrification of ammonia and ammonium salts at the surface of various photocatalysts present in the soil such as aluminum or titanium oxides. Batham and Nigam (6) believe solar activity is the most important factor in controlling the activity of nitrifying bacteria in soils. Waksman and Madhok (115) and Fraps and Sterges (33) conclude from their studies that the formation of nitrate in the soil definitely is due to biological activity and not to sunlight.

During the period of 1900 to 1920 many nitrification studies were made, both in soil and liquid media. In almost all of these studies a nitrogen compound of some type was added as a source of material for the micro-organisms to nitrify (14, 15, 16, 37, 74, 88). One per cent bloodmeal or 100 milligrams ammonium sulphate in 100 grams of soil was used most often. Cottonseed meal and other organic nitrogen materials were also used. Lipman and Burgess (73) tested 14 different forms of nitrogen fertilizer of which 12 were organic materials for their effect on nitrification when added to 29 different soil types. They concluded that field experiments were beginning to give strong evidence that relative availabilities of different forms of nitrogen in fertilizers, as determined by nitrification in the laboratory, gave reliable indices to the actual relationships between these different forms of nitrogen in the field. Brown (15) found nitrification was higher with dried blood than with ammonium sulphate. Burgess (18) studied nitrification on nine Hawaiian soils, three of which were high, three medium, and three low in productivity. He used three forms of nitrogen, alfalfa meal, dried blood and fish scrap which were added to 100 grams of soil at the rate of 30 milligrams of nitrogen. From the results obtained he concluded that nitrification is by far the most accurate biological soil test yet outlined for predicting soil fertility.

In 1915, Allen and Bonazzi (1) questioned the methods being used in studying nitrification. They emphasized that it would be impossible to solve the complex problem encountered with methods so frought with errors. A year later Kelley (60, 61) showed certain soils were capable of supporting active nitrification of one per cent bloodmeal while others were not. The results varied enormously when different concentrations were used. The nitrate production varied among soils with the same concentration of bloodmeal. The length of incubation also greatly influenced the results. In nitrification studies in the laboratory, Kelley emphasized the necessity of approximating field conditions. A few years later, Waksman (112, 113) showed the nitrification of blood-meal or ammonium sulphate is not a good test for comparing different soils. In the oxidation of ammonium sulphate, both nitric and sulphuric acids are formed. The hydrogen ion concentration may be increased to the point where nitrification is diminished or ceases. Less injury will be sustained in the highly buffered soils. The amount of nitrate that accumulates when nitrogenous materials are added to the soil will depend on the initial reaction of the soil, the buffer capacity, and final reaction more than on biological activities.

Experimental

Plan of procedure

In developing or adapting a procedure for soil testing it is essential to know the influence exerted upon the results by various factors. In nitrification rate studies, the size of sample, type of container, per cent moisture, aeration, temperature, and granular size of soil are some of the factors that must be considered. A preliminary study was made to evaluate some of these factors before more comprehensive tests were undertaken.

The phenoldisulfonic acid method for determining nitrate has been used for over 30 years and although several factors may influence the results, it is still one of the most accurate and popular procedures (23, 51, 94). The procedure most often used, which is outlined by Prince (94), is too time consuming for use in a soil testing laboratory. Studies were undertaken to find modifications which would speed up the analysis without sacrificing accuracy. This included use of a photometer as outlined by Berge (8).

Factors affecting nitrification

For nitrification studies a large, well insulated ice box with glass sidewalls was made over into an incubator. Heavy wire shelves were spaced about eight inches apart. Unglazed earthen pans about 12 inches in diameter and an inch deep were placed on the top and bottom shelves and filled with water to keep the atmosphere nearly saturated. A network of resistance wires connected with a thermoregulator was mounted inside the box. Temperatures could be controlled to within $\pm$ 1.0° C. Figure 1 shows the lower portion of the improvised incubator with the water pans and incubation bottles.

Figure 1. Lower portion of an incubator constructed from an old refrigerator

In the preliminary studies on nitrification a soil was selected that had a pH of 6.6, was medium in organic matter and silt loam in texture. Sufficient distilled water for 25 per cent moisture was added to the soil and after thorough mixing on a glazed paper, the samples were transferred to pint milk bottles or 300 ml Erlenmeyer flasks. The volume was reduced by striking the bottom of the container on a large rubber stopper until the desired compaction was obtained. The containers were weighed two or three times a week and moisture added as needed.

Preliminary studies indicated nitrification rates could be determined as accurately with 25 gram soil samples as with 50 or 100 gram samples in either pint milk bottles or 300 ml Erlenmeyer flasks. These studies were expanded to include nitrification rates using 25 grams of soil in 50 ml beakers, and 10 grams of soil in 30 ml wide mouthed bottles for comparison with 25 and 100 gram samples in pint milk bottles. In all of these studies the samples were wetted to 25 per cent moisture with a fine spray and incubated at 35° C.

For nitrate determination, the 30 ml bottles were assembled in trays as shown in Figure 2. These trays were so constructed that 12 bottles were held firmly in position but could be readily removed by releasing the two hooks holding the top in place. Water was added to the 12 bottles in one

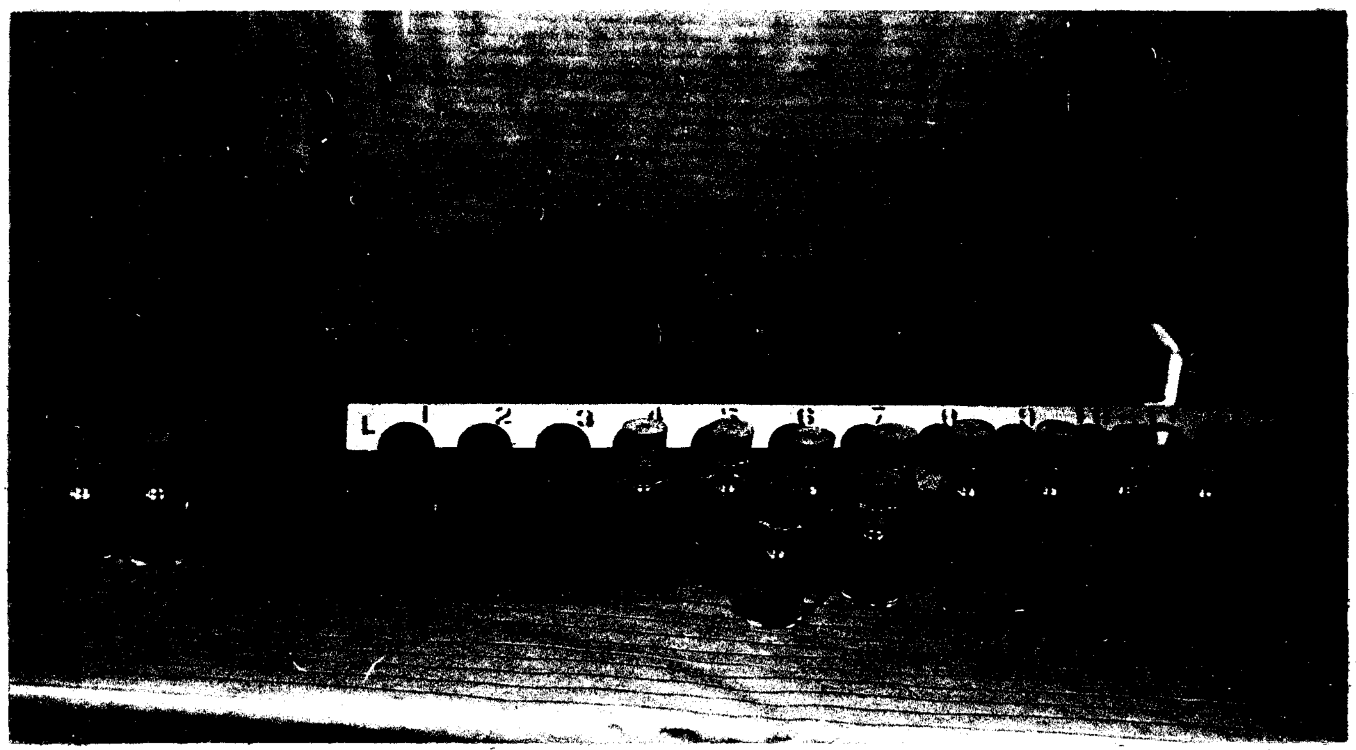

Figure 2. Small bottles are placed in trays for shaking with water and CaO to remove nitrate

operation with the dispensing apparatus shown in Figure 3. To filter the soil from the extraction solution, the extraction trays were inverted over the filtering trays. The ends of the extraction tray rest on the groove or shelf on the inside of the two ends on the filtering tray (Figure 4). This stabilizes the extraction tray and assures pouring into the funnel tubes without spilling the solution.

The majority of surface soils taken from cultivated fields in Iowa vary from a loam to silty clay loam in texture and contain from about three to six per cent organic matter. Six representative soils were selected for comparison of the efficiency of one hole rubber stoppers and cheesecloth plugs in preventing loss of moisture from the soil samples during incubation. The optimum moisture content for nitrification was studied also. Characteristics of the soils were as follows:

Sample 1	loam	medium organic matter
Sample 2	silt loam	medium to low in organic matter
Sample 3	silt loam	high in organic matter
Sample 4	silty clay loam	medium high in organic matter
Sample 5	silty clay loam	high in organic matter
Sample 6	silty clay loam	medium in organic matter

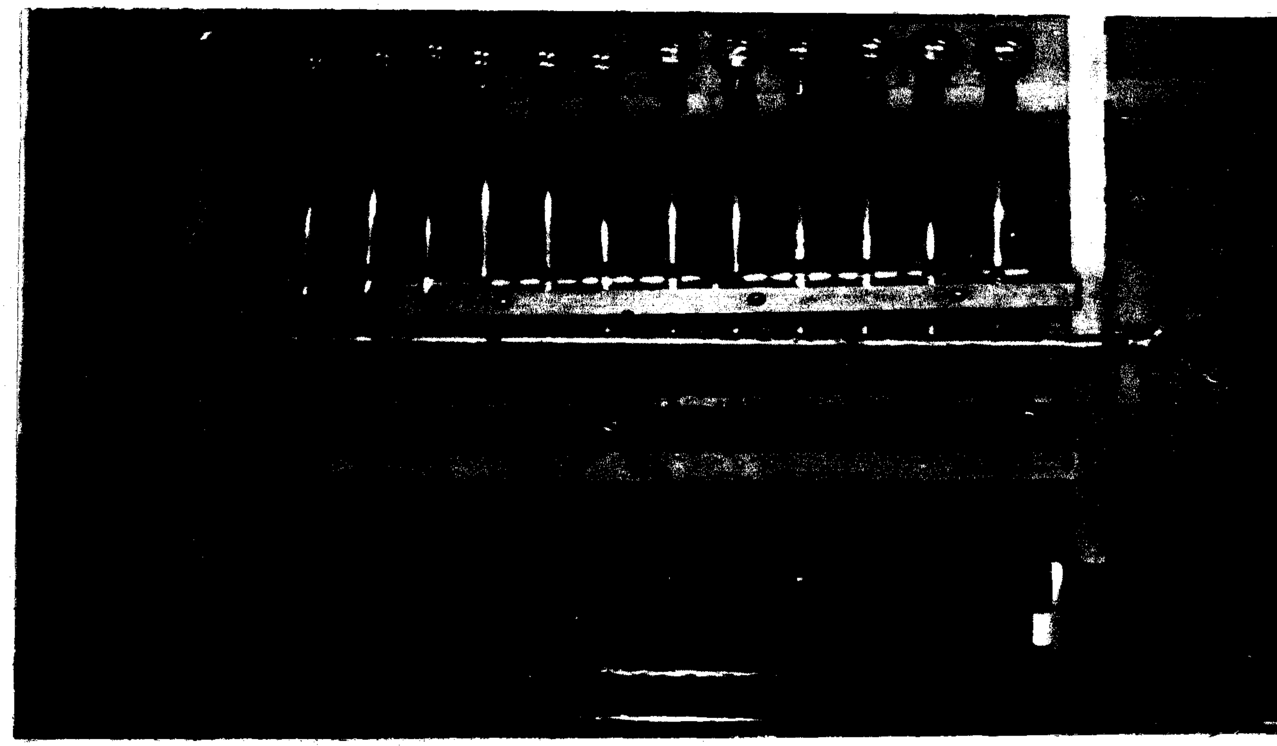

Figure 3. Dispensing apparatus for adding 20 ml of water to each of 12 bottles in one operation

Figure 4. Filtering 12 samples at once after shaking mechanically

All samples ranged from pH 6.0 to 7.0 and according to soil test results were medium-high in available phosphorus and potassium.

In studies of the influence of moisture on nitrification, 25 gram samples of soil were adjusted to 15, 20, 25 and 30 per cent moisture. Samples were wetted with a fine spray of distilled water, placed in pint milk bottles and covered with one hole rubber stoppers or cheesecloth plugs. Incubations were carried out at 35° C for periods of two and three weeks. The bottles were weighed at the beginning of the experiment, and again at the end of the two or three week incubation periods.

Obtaining a uniform distribution of water and air throughout the soil mass when adding various increments of water is a difficult if not impossible task. In order to obtain a more uniform distribution of both moisture and air in determining the effect of moisture upon nitrification, 10 gram soil samples were first saturated with water. Then the excess water was removed by placing the samples in a pressure membrane apparatus under 1, 2 and 5 atmospheres of pressure and on a porous ceramic plate under 50 and 100 cm of water tension. The soils on the tension plate were wetted to saturation by reversing the process and permitting water to enter the tube containing the soil from the bottom. In this way puddling of the samples was avoided. After moisture equilibrium was reached under the various pressures or tensions, the samples were transferred to 30 ml bottles, stoppered with one hole rubber stoppers and incubated at 35° C for three weeks.

To further study the effect that containers might have upon nitrification in relation to air space, 14 samples, all silt loam in texture and medium in organic matter, were incubated in pint milk bottles and in 50 ml beakers. Twenty-five gram samples were placed in both containers and wetted to 25 per cent moisture. One hole rubber stoppers were placed in both containers and the samples were incubated at 35° C for three weeks.

Several workers have shown that nitrification is greatly retarded at temperatures above 45° C. Thompson (110), however, obtained high mineralization of nitrogen within a week's time at 60° and 70° C. He did not separate ammonia from nitrate nitrogen and no doubt most of the mineralization was ammonia released by thermophilic micro-organisms. Since nitrate is formed by oxidation of ammonia, studies were undertaken to determine the effect of varying temperatures upon nitrification rates. Ten soil samples, taken from the rotation plots at Ames, were selected for the study. The 25 gram portions of soil were placed in pint milk bottles and adjusted to 25 per cent moisture. Group 1 was incubated for three weeks at 35° C. Group 2 was incubated for three days at 55° C followed by incubation for 18 days at 35° C. Group 3 was incubated for seven days at 55° C followed by incubation for 14 days at 35° C, and Group 4 was incubated for only seven days at 55° C.

Two studies were undertaken to determine the influence of the soil aggregate size upon nitrification. For the first study two composite samples were taken from a Clarion silt loam soil near Ames. Field run is designated as soil thoroughly mixed by hand at field moist condition so all clods are reduced to less than one-fourth to three-eighths inch in diameter. The soils were then air dried by large fans. After drying a portion of each sample was crushed and separated by screens into two aggregate sizes, that greater than 20 mesh but less than 10, and that less than 20 mesh. Twenty-five grams of each fraction were placed in pint milk bottles, adjusted to 25 per cent moisture and incubated at 35° C for three weeks.

Twelve samples from the rotation plots at Clarinda were used in the second study. Field run soils were compared with samples crushed to pass a 10 mesh screen. To better sample the larger aggregate size of soil and the undecomposed plant residues, 100 gram samples were used. They were placed in pint milk bottles, moistened to 25 per cent moisture and incubated at 35° C for three weeks.

Modifications of phenoldisulfonic acid method

In the usual procedure of determining nitrate nitrogen in soils by the phenoldisulfonic acid method, two to four grams of powdered calcium oxide are added to 100 grams of soil before shaking with 100 or 200 ml of water. The samples are intermittently shaken for one hour by hand before filtering. The calcium oxide flocculates the soil and aids in obtaining a clear filtrate. A 10 ml portion is evaporated to dryness on a steam bath. Then 2 ml of phenoldisulfonic acid are added to the residue and after 10 minutes 15 ml of water and 12 to 15 ml of 1:1 ammonium hydroxide are added. The solution is transferred to a 100 ml volumetric flask or 100 ml graduate cylinder and made up to volume with distilled water. The intensity of the yellow color produced by formation of ammonium nitrophenoldisulfonic acid is compared with standard nitrate solutions.

One of the slower parts of the procedure as outlined above is removal by a pipette of 10 ml of filtrate, evaporating to dryness and then making up to 100 ml volume after addition of ammonium hydroxide. To speed up the procedure, trials were made in which 2 ml portions of filtrate were removed by an automatic pressure bulb pipette and transferred to 50 ml beakers for evaporation (Figure 5). The phenoldisulfonic acid was added with a similar pipette. Portable automatic pipettes as described by Smith (107) were used to add 12.5 cc of water and 6.5 cc of ammonium hydroxide. The ammonium hydroxide was applied under pressure and mixed the solutions which eliminated the necessity of stirring. The total volume of solution was 20 ml. The proportion of the 2 ml extract evaporated to dryness and solutions added to total 20 ml was the same as 10 ml made up to 100 ml in volume.

Loss of nitrate may occur during evaporation if the filtrate becomes acid. This will be true especially near the end of the evaporation process (23). Sufficient calcium oxide must be added to insure a clear filtrate, alkaline in reaction without adding so much that interference with nitrate determination will result. Sufficient ammonium hydroxide must be added at the end of the procedure to insure an alkaline reaction with full development of the yellow color. A 2 x 2 x 2 factorial experiment using 10 grams of soil with two quantities each of calcium oxide, phenoldisulfonic acid and ammonium hydroxide was run to determine the effect on nitrate analysis. Calcium oxide was added in quantities of 0.1 and 0.4 grams before the addition of 20 ml of water for

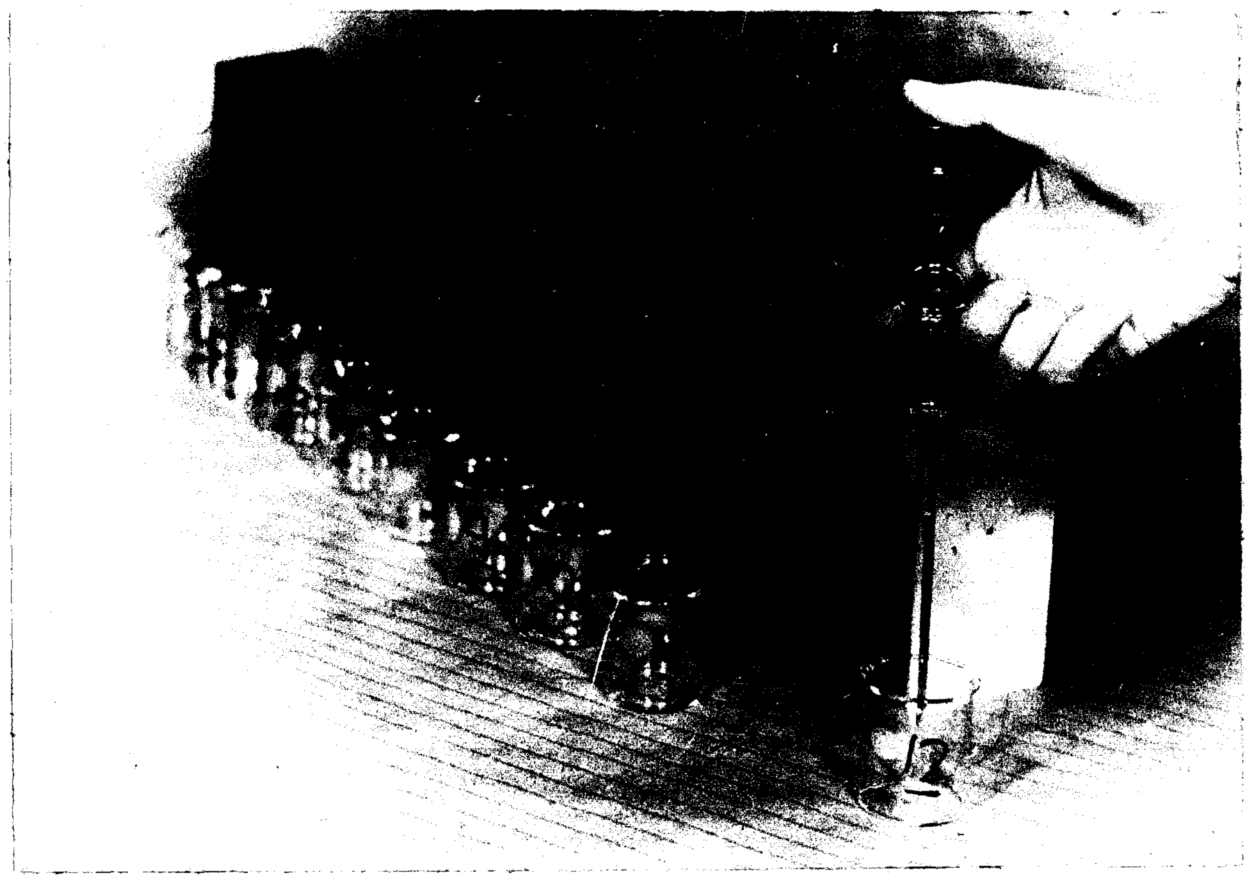

Figure 5. Transferring 2 ml of filtrate from filter tubes to 50 ml beakers with pressure bulb pipette

nitrate extraction. After evaporation of 2 ml aliquots of the filtrate, 0.5 ml and 1.0 ml of phenoldisulfonic acid was added respectively to separate samples. The two rates of 1:1 ammonium hydroxide were 6.5 ml and 13.0 ml.

To investigate the influence of shaking and stirring upon the removal of nitrate nitrogen from the soil, two studies were conducted. In the first study 10 gram samples were employed in 30 ml bottles and in the second 25 and 50 gram samples employed in pint milk bottles. In the 30 ml bottles, 20 ml of water and 0.15 grams of calcium oxide were added to the 10 grams of soil. The samples were allowed to stand 10 minutes, 30 minutes and 60 minutes with and without stirring with a glass rod. At the end of the periods the samples were shaken for 10 minutes on a specially built platform shaker which holds four trays of 12 bottles each. The lid was lined with sponge rubber covered with plastic cloth (Figure 4). Ordinary trunk locks on the front of the shaker pressed the lid tight over the bottles in the extracting trays and prevented leakage. A damp towel was used to wipe off the plastic covering after the shaking was completed. An automatic time clock controlled the shaking period.

The 25 and 50 gram samples were placed in one pint milk bottles. Then 100 ml of water and 0.50 gram calcium oxide were added. The samples were placed on an International bottle shaker for periods of 5, 10 and 30 minutes. Other samples were shaken intermittently for one hour by hand.

The intensity of the yellow color was measured on a Klett-Summerson photometer. Instead of testing standard solutions with each set of determinations, a calibration curve for the instrument was made using 0.05, 0.10, 0.20, 0.25, 0.30, 0.40 and 0.50 milligrams nitrate nitrogen.

Results

Factors affecting nitrification

The results of the preliminary study on sample size, container and length of incubation period are shown in Table 1 and Figure 6.

Table 1. The effect of sample size, container and length of incubation upon nitrification

Time of incubation	Nitrate nitrogen production - ppm					
	25 grams		50 grams		100 grams	
	flask	bottle	flask	bottle	flask	bottle
1 week	42.0	42.0	45.0	41.0	41.0	43.0
	44.0	42.0	45.0	39.0	46.4	45.0
2 weeks	72.0	71.2	63.0	65.0	60.0	65.0
	71.2	68.0	64.4	64.8	60.0	65.0
4 weeks	84.0	74.0	73.0	71.0	69.0	69.0
	84.0	78.0	76.0	70.0	68.0	67.0

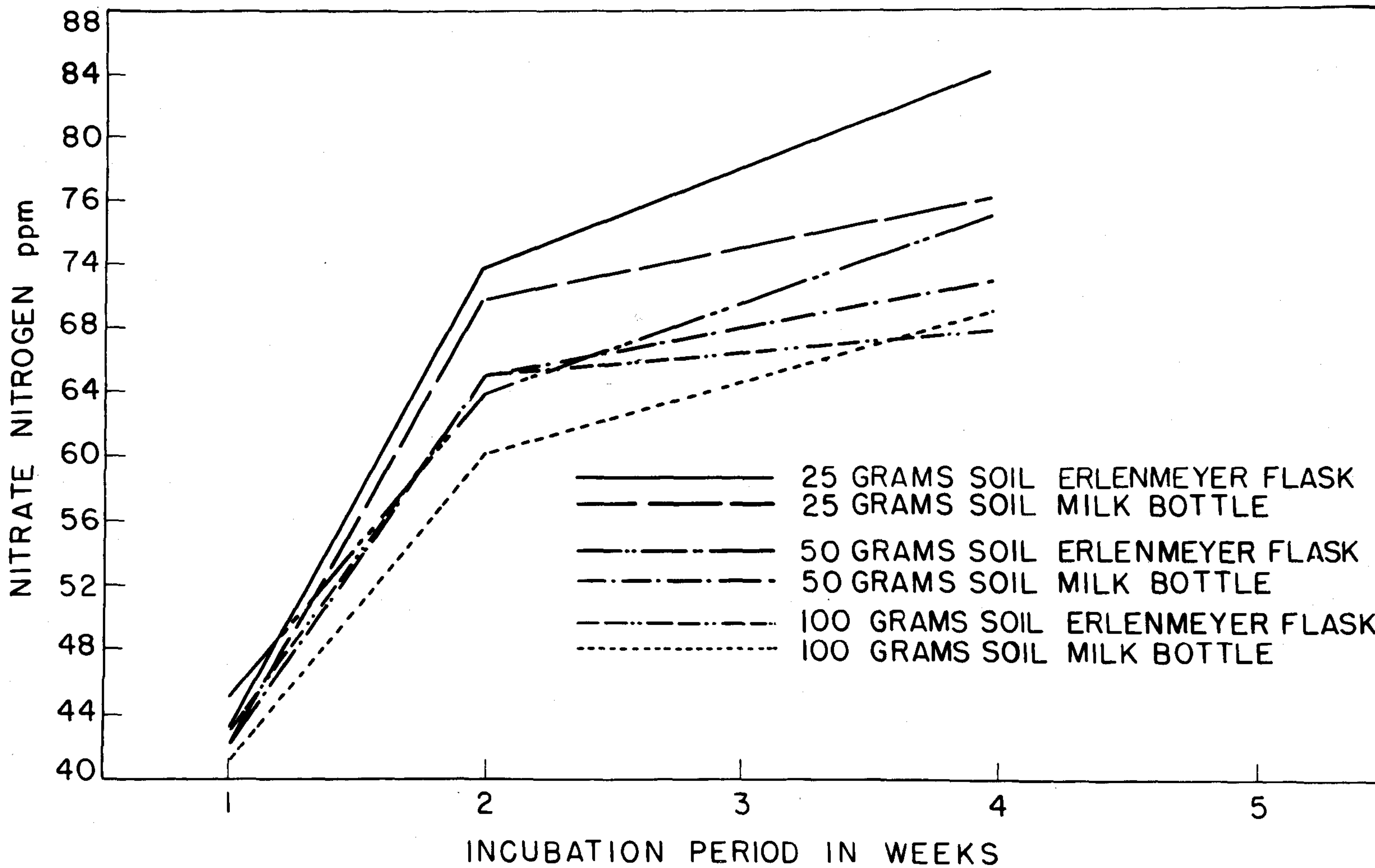

Figure 6. Nitrate production during 1, 2, and 4 week intervals using 25, 50, and 100 gram samples of soil

In general the variation in duplicate samples is no greater than that usually obtained in nitrification studies.

The type of continer appears to have little or no effect as long as the moisture is kept constant and the compaction is the same. In the 25 gram samples, the nitrate was slightly higher in the Erlenmeyer flasks while the reverse was true with the 100 gram samples. The reason for this probably was due to evaporation. The milk bottles lost moisture by evaporation a little more rapidly than did the flasks.

No difference in nitrification was obtained during the first week of incubation in regard to sample size or container. In the two- and four-week incubation periods, slightly more nitrate was found in the 25 gram samples than either the 50 or 100 gram samples. This was true for both the flasks and milk bottles although the difference was not so great in the milk bottles. These differences possibly can be explained by the relative loss of moisture between sizes of samples and by the amount of aeration.

The loss of moisture from soil samples, when the bottles were stoppered with a few folds of cheesecloth, was sufficiently large during incubation in a nearly saturated atmosphere to influence nitrification (Table 2). Some soils lost moisture more readily than others but no relationship could be established between texture and organic matter content with evaporation. There appeared to be a tendency, however, for soils highest in initial moisture to lose water at the most rapid rate. Variations in moisture losses might be explained by differences in aeration due to puddling.

Table 2. Loss of moisture from 25 grams of soil in one pint milk bottles during incubation in a nearly saturated atmosphere at 35° C when stoppered with cheesecloth

Soil sample	% moisture after 2 weeks				% moisture after 3 weeks			
	15*	20*	25*	30*	15*	20*	25*	30*
1	10.4	12.8	18.8	22.4	1.0	4.0	8.0	15.2
2	8.0	16.4	20.8	24.0	1.2	10.0	6.0	16.0
3	10.0	13.2	21.6	18.8	6.0	9.2	14.0	9.2
4	6.0	14.4	14.0	16.0	2.0	5.2	2.0	14.8
5	9.2	11.2	18.4	24.4	4.4	6.4	8.8	19.6
6	10.0	13.6	21.2	23.6	2.0	11.2	14.8	22.0

*Per cent moisture initially.

Pint milk bottles stoppered with one hole rubber stoppers lost little or no moisture during two- or three-week incubation periods when the samples were placed in a nearly saturated atmosphere. After the procedure of using one hole rubber stoppers to cover the bottles was adopted, moisture maintenance was determined by weighing bottles at the start and end of three weeks incubation on 316 samples. The containers included pint milk bottles, 50 ml beakers and 30 ml bottles with 100, 25 and 10 gram samples of soil. The loss in weight was less than 0.5 gram in all instances but two where the loss was approximately 1.0 gram.

Nitrification takes place over a rather wide range of moisture as indicated in Table 3. Thorough mixing of soil samples with water to obtain a uniform distribution of moisture and air is difficult. No doubt this is responsible to a large degree for variation between duplicate determinations.

Table 3. Effect of moisture upon nitrification during 2 weeks incubation at 35° C

Soil moisture per cent	Nitrate nitrogen production - ppm					
	Soil sample number					
	1	2	3	4	5	6
15	40.0	36.8	44.0	54.0	64.0	27.6
	32.0	45.2	44.4	61.6	63.2	32.0
20	34.0	47.6	40.0	47.2	70.4	35.2
	28.4	34.0	37.6	64.0	87.2	34.0
25	38.8	34.0	44.0	64.0	92.4	48.8
	34.2	34.0	46.2	68.0	89.2	44.2
30	32.8	44.0	38.8	70.4	87.2	29.6
	21.2	44.0	44.0	70.4	89.2	48.0

Greatest variation between duplicate determinations occurred in the 15 and 30 per cent moisture levels. Small pockets of soils too dry or too wet probably occurred at these moisture percentages. The field carrying capacities and physical condition of the soil samples would influence the results. In general nitrification increased to some extent with increase in moisture. Most uniform results between duplicate samples and highest nitrification rates were obtained on the 25 per cent moisture level.

A more definite trend in the effect of soil moisture upon nitrification, as shown in Table 4, was found where moisture was more uniformly distributed through the soil mass by means of pressure or tension.

Nitrification was highest in all instances where moisture was adjusted to 100 cm of water tension. From 30 to 35 per cent moisture appears to be optimum where the water is uniformly distributed throughout the soil mass and where the physical conditions are not greatly altered in the wetting up process. The moisture content of samples 1 and 4 was too high for nitrification at 50 cm water tension.

The practice of wetting the soils to 25 per cent moisture corresponds closely to the moisture content at one atmosphere pressure on the four samples studied. The per cent moisture at one atmosphere is regarded as being near the field carrying capacity of soils. Several workers have suggested the field carrying capacity as being optimum for nitrification.

Nitrification was slightly higher with all 14 samples in the 50 ml beakers as compared to pint milk bottles (Table 5).

Table 4. Effect of moisture upon nitrification during 3 weeks incubation at 35° C

Pressure or tension	Soil sample 1		Soil sample 2		Soil sample 3		Soil sample 4	
	% H_2O	NO_3-N	% H_2O	NO_3-N	% H_2O	NO_3-N	% H_2O	NO_3-N
5 atmos. pressure	18.3	16.7	15.8	23.8	20.5	29.0	14.8	25.1
2 atmos. pressure	19.8	17.7	15.4	23.2	20.0	26.8	15.7	25.2
1 atmos. pressure	25.5	27.7	22.8	35.5	27.1	36.8	22.8	28.1
Regular procedure	25.0	28.2	25.0	25.4	25.0	35.8	25.0	26.4
100 cm H_2O tension	37.3	48.2	25.4	55.6	30.7	49.6	34.4	46.1
50 cm H_2O tension	41.0	2.3	30.0	53.9	35.9	49.6	40.9	12.7

Table 5. Influence of containers upon nitrification rates

Soil sample	Nitrate nitrogen production	
	Pint milk bottle	50 ml beaker
1	44.6	47.2
2	39.6	43.4
3	36.0	37.6
4	32.0	36.4
5	50.2	51.8
6	51.6	54.4
7	48.2	49.6
8	54.6	58.0
9	49.2	51.2
10	51.6	55.6
11	43.8	47.2
12	46.4	47.6
13	39.2	42.8
14	43.8	48.0

Analysis of variance table

Source	d.f.	S.S.	M.S.
Treatment	1	114.29	114.29
Samples	13	2123.99	163.38
Samples x treatment	13	16.35	1.26
Between duplicates	28	42.96	1.53
Total	55	2297.59	

The variance is no greater than between samples as indicated in the analysis of variance table but the difference is consistent. Variance between duplicate determinations with either container was negligible.

The influence of sample size upon nitrification is shown in Table 6. The mean nitrification of 100 gram and 25 gram samples for the 19 soils was identical (61.2 ppm). The mean nitrification rate of the 10 gram samples was 54.3 ppm or 6.9 ppm less than was found in the 100 or 25 gram samples. Although the results obtained with the 10 gram samples are significantly lower than the larger samples, any one of the three sample sizes can be used with equally good results as long as they are employed throughout the study.

The effect of incubating the soils at a relatively high temperature on nitrification is shown in Table 7. Incubation of soil samples for only three days at 55° C was effective in destroying nitrifying bacteria. The amount of nitrate in the soil at the end of seven days incubation at 55° C is the same as in the soils incubated three days at 55° C followed by 18 days at 35° C or seven days at 55° C followed by 14 days at 35° C. If ammonia nitrogen is released by thermophilic organisms, it is apparent that the nitrifying organisms have either been destroyed by this temperature or were unable to start multiplying again within the 18 day incuba-

Table 6. A comparison of sample size and containers upon nitrification rates

Sample number	Initial nitrate	Nitrate nitrogen production		
		100 gram*	25 gram*	10 gram*
16-1	3.2	62.8	64.6	55.8
16-2	3.4	57.3	58.9	53.3
16-3	3.4	56.5	59.3	47.7
63-1	3.5	58.5	59.3	50.6
63-2	3.4	52.3	58.6	52.0
63-3	3.8	59.0	59.1	50.8
96-1	3.1	59.0	66.1	52.2
96-2	3.0	57.8	60.6	49.8
96-3	3.3	59.8	66.5	48.6
20-1	2.2	55.8	55.9	47.4
20-2	2.8	53.0	54.8	44.6
20-3	2.3	53.5	53.0	45.9
1811	5.4	59.3	60.8	52.8
1812	8.4	55.0	52.3	50.4
1815	12.4	56.5	49.3	46.9
1816	4.0	57.0	62.1	49.2
1818	40.0	118.3	116.9	107.1
5x	8.8	65.3	53.4	63.7
5y	15.6	66.0	51.2	63.2
Mean		61.2	61.2	54.3

*The 100 and 25 gram samples were incubated in pint milk bottles, the 10 gram sample in 30 ml bottles.

Table 6 (Continued)

Analysis of variance table

Sources	d.f.	S.S.	M.S.
Methods	2	599.39	299.70
100 vs. 25 gram	1	0	
(100 + 25) vs. 10	1	599.39	599.390
Sample	18	10215.54	567.530
Methods x samples	36	581.94	16.165
Total	56	11396.87	

Fiducial limits at 5% level = 6.87 ± 2.03 x 1.304 = 9.52 and 4.22.

Table 7. The influence of 55° C temperature for a short time upon nitrification in soils

Sample number	Initial NO_3-N ppm	Nitrate nitrogen production - ppm			
		Group 1*	Group 2*	Group 3*	Group 4*
3A-1	2.8	45.4	7.2	6.8	6.8
3C-1	2.9	44.2	6.8	5.6	6.0
3D-1	2.9	45.2	7.2	5.6	5.8
4A-1	3.9	41.2	7.2	7.6	8.0
4C-1	4.2	42.4	8.8	8.0	8.2
4D-1	3.9	41.0	6.8	7.6	7.4
7B-1	4.5	69.2	8.4	10.0	7.6
7E-1	4.3	63.4	10.0	10.0	9.6
6A-1	3.1	48.0	9.2	8.8	8.0
6C-1	4.1	43.6	8.8	5.6	6.6

*Group 1 - incubated 3 weeks at 35° C.
Group 2 - incubated 3 days at 55° C followed by 18 days at 35° C.
Group 3 - incubated 7 days at 55° C followed by 14 days at 35° C.
Group 4 - incubated 7 days at 55° C.

tion period. The amount of nitrate formed was much less than in the samples incubated three weeks at 35° C.

Nitrification of soils in relation to aggregate size is shown in Tables 8 and 9. In the first study there was as much variability between duplicate determinations as between treatments. In the second study, where a larger number of samples were included in the experiment, the nitrification rate is consistently higher on the soils crushed to pass the 10 mesh screen in comparison to the field run. The mean difference is only about 2.0 ppm which is within the normal limit of experimental error in nitrification studies. The greatest difference between treatments on any of the samples was less than 5.0 ppm. This indicates that aggregate size has no marked influence upon the nitrification processes.

Table 8. Nitrification of soils in relation to aggregate size

Sample number	Nitrate nitrogen production - ppm		
	Field run	10 to 20 mesh	Less than 20 mesh
1	39.6	38.8	41.6
1	48.4	42.0	43.2
2	55.2	50.8	57.2
2	54.4	49.2	58.8

Table 9. Nitrification of soils in relation to aggregate size

Sample number	Nitrate nitrogen production - ppm		
	Field run	Less than 10 mesh	Difference
16-1	47.8	50.2	+ 2.4
16-2	43.0	45.8	+ 2.8
16-3	40.2	45.2	+ 5.0
63-1	45.0	46.8	+ 1.8
63-2	40.8	41.8	+ 1.0
63-3	44.0	47.2	+ 3.2
96-1	49.6	47.2	- 2.4
96-2	46.8	46.2	- 0.6
96-3	45.6	47.8	+ 2.2
20-1	43.4	44.6	+ 1.2
20-2	39.2	42.4	+ 3.2
20-3	41.4	42.8	+ 1.4
Mean	43.9	45.7	+ 1.8

Analysis of variance table

Source	d.f.	S.S.	M.S.
Samples	11	159.39	14.49
Treatments	1	18.72	18.72
Samples x treatment	11	20.12	1.83
Total	23	198.23	

Shown in Table 10 is a comparison of two procedures for developing the yellow color in the phenoldisulfonic acid method of nitrate analysis. In the first procedure, a 2 ml aliquot of filtrate was evaporated to dryness and then made to 20 ml volume by addition of measured quantities of phenoldisulfonic acid, water, and ammonium hydroxide. In the second procedure, a 10 ml aliquot of filtrate was evaporated and after adding the acid and ammonium hydroxide, it was brought to 100 ml volume in a volumetric flask. The results with the 2 ml aliquot are consistently higher than the 10 ml portion but the difference is not great. No doubt, a slight difference in the technique of measurement is responsible. The variability between duplicate determinations is not large and the results indicate either procedure can be used with an equal degree of accuracy.

The results of the 2 x 2 x 2 factorial experiment using two rates of calcium oxide, phenoldisulfonic acid and ammonium hydroxide are shown in Table 11. Variance of calcium oxide from 0.1 to 0.4 grams, phenoldisulfonic acid from 0.5 to 1.0 ml or ammonium hydroxide from 6.5 to 13.0 ml had no appreciable effect upon the nitrate removed from 10 grams of soil. Apparently 0.1 gram of calcium oxide was sufficient to flocculate the soil and keep the filtrate alkaline during evaporation. Accuracy in measuring the phenoldisulfonic acid

Table 10. A comparison of size of aliquots and dilution in nitrate determination

Sample number	Nitrate nitrogen - ppm 2 ml portion	10 ml portion	Difference
F-962	89.2	88.0	+ 1.2
F-962	88.0	86.0	+ 2.0
F-963	86.0	81.6	+ 4.4
F-963	88.0	82.4	+ 5.6
F-965	81.6	82.4	- 0.8
F-965	84.8	83.6	+ 1.2
F-966	30.8	29.2	+ 1.6
F-966	33.6	32.0	+ 1.6
F-1013	80.4	74.8	+ 5.6
F-1013	77.2	74.8	+ 2.4
F-1014	98.0	94.8	+ 3.2
F-1014	98.8	94.8	+ 4.0

Analysis of variance table

Source	d.f.	S.S.	M.S.
Samples	5	10692.48	2138.50
Treatment	1	42.67	42.67
Samples x treatment	5	16.45	3.29
Between duplicates	12	24.16	2.01
Total	23	10775.76	

Table 11. The effect of varying calcium oxide, phenoldisulfonic acid and ammonium hydroxide upon nitrate analysis

Treatment			Nitrate nitrogen
Calcium oxide	Phenoldisulfonic acid	Ammonium hydroxide	
(grams)	(ml)	(ml)	(ppm)
0.1	0.5	6.5	10.0
0.1	0.5	13.0	10.0
0.4	0.5	6.5	10.0
0.4	0.5	13.0	9.4
0.1	1.0	6.5	10.4
0.1	1.0	13.0	10.4
0.4	1.0	6.5	10.0
0.4	1.0	13.0	9.2

or ammonium hydroxide was not critical other than to obtain an accurate volume of the final solution.

The results of experiments on removal of nitrate from soil are shown in Tables 12 and 13. In determining nitrate in 10 grams of soil with 20 ml of water in small bottles it was found that stirring the soil with a glass rod before shaking was essential. The small bottles were too full to obtain a thorough mixing of water, calcium oxide and soil without the initial stirring. When placed on the platform shaker without stirring, the soil and water had a tendency to rock with motions of the shaker rather than becoming thoroughly mixed. Stirring with a glass rod was found to be

Table 12. The effect of stirring and length of wetting period upon nitrate analysis

Sample number	Nitrate nitrogen - ppm					
	10 min.	10 min. + stir	30 min.	30 min. + stir	60 min.	60 min. + stir
16x	16.2	20.0	19.2	23.0	15.6	20.0
16x	15.6	21.0	19.2	23.0	18.0	21.0
18x	3.0	4.2	3.5	4.2	4.2	4.5
18x	4.0	4.2	4.0	4.5	4.0	4.2

even more essential on soils that had been incubated for three weeks than on the initial soil analysis.

The time to allow the samples to stand with water and calcium oxide before placing on the shaker, apparently had little effect on nitrate removal. For some unknown reason nitrate removed in the 30 minute period was slightly higher than the 10 or 60 minute periods on sample 16x.

All of the nitrate in 25 or 50 grams of soil was dissolved by 100 ml of water when the pint milk bottles were shaken for only 5 minutes on an International bottle shaker (Table 13). There was no significant difference in the amount of nitrate removed from 5, 10 or 30 minutes of shaking as compared to 60 minutes of intermittent shaking by hand. Nitrate is very soluble in water and in the vigorous shaking the samples received on the bottle shaker all of the nitrate was dissolved within the 5 minute period.

Table 13. The removal of nitrate from soil in relation to time of shaking

Sample number	Weight grams	Nitrate nitrogen - ppm			
		5 min.	10 min.	30 min.	60 min. (by hand)
1	25	9.9	11.0	8.8	8.2
	25	9.6	10.0	9.2	9.2
	50	9.0	8.6	8.0	7.9
	50	9.4	9.6	8.0	8.6
2	25	30.8	28.0	29.4	28.8
	25	28.4	29.0	28.4	29.6
	50	25.8	25.7	26.6	26.2
	50	26.4	27.0	25.8	27.0

In preparation of a calibration curve for the photometer standard solutions containing 0.05, 0.10, 0.20, 0.25, 0.30 and 0.40 milligrams nitrate nitrogen were made using potassium nitrate. The readings obtained from these standard solutions on a Klett-Summerson photometer are shown on the graph in Figure 7. Since the photometer scale is logarithmic on this instrument, a straight line was obtained. A change of 10 divisions on the photometer scale corresponds to 0.0055 milligrams nitrate nitrogen. Duplicate samples of standard solutions can be determined with an accuracy of less than five divisions. Standard solutions containing 0.05 and 0.10 milligrams nitrate nitrogen were checked periodically to make certain the curve for the instrument remained the same.

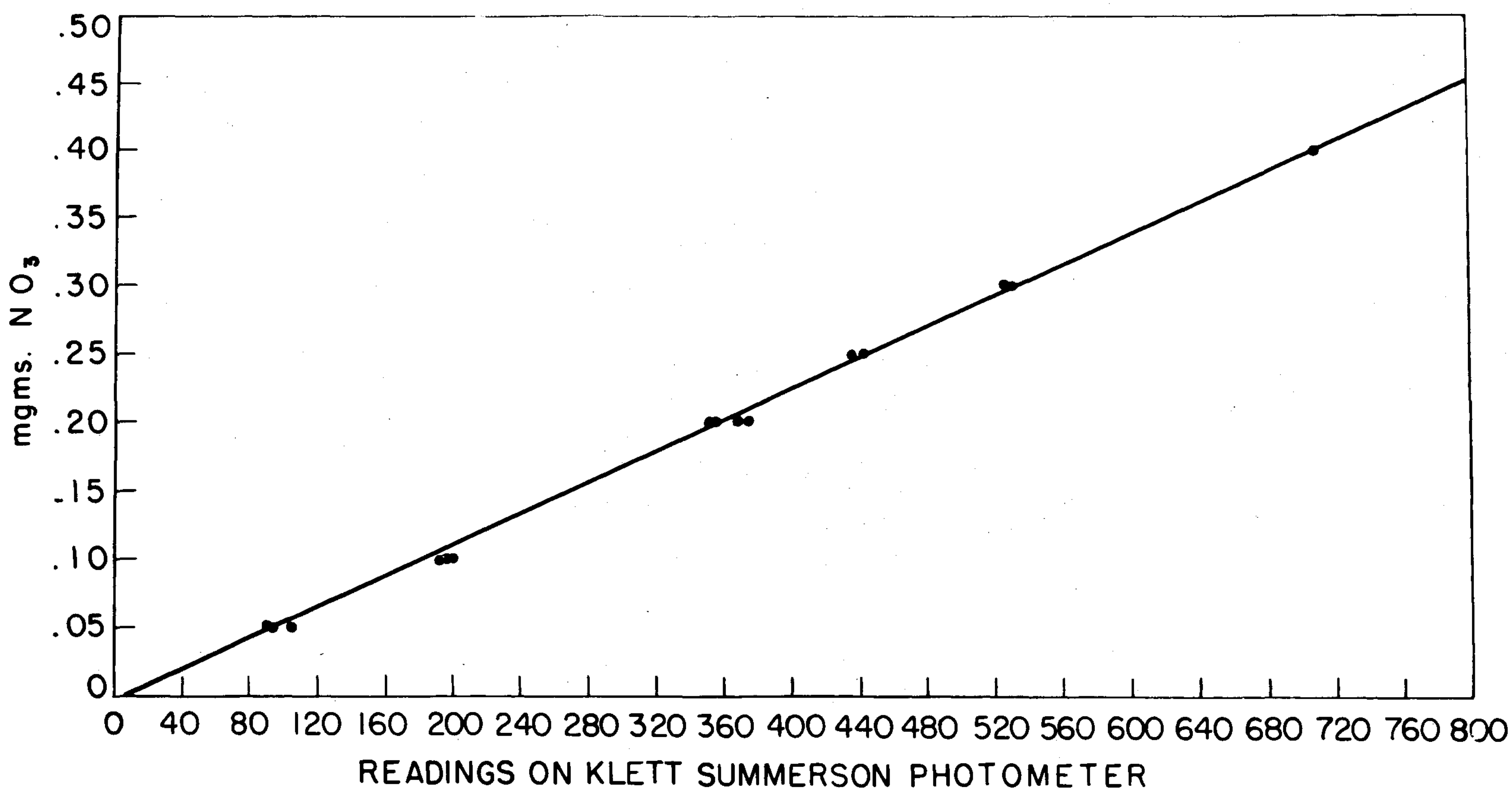

Figure 7. Nitrate calibration curve for a Klett Summerson photometer

Standard solutions were always determined when new solutions of phenoldisulfonic acid were prepared.

Discussion

Soil moisture and aeration have been shown to be very important factors in nitrification by many research workers. In 1915, Allen and Bonazzi (1, p. 35) criticized the methods being used for nitrification rate studies. The following paragraphs are taken from their publication.

> The first method of procedure with which we must take issue is the maintenance of moisture content of the sample, either by weighing the container and sample at stated intervals and replacing the loss, or by preventing loss by plugging the container with moistened absorbent cotton. In the former case, aside from the large amount of experimental work involved, there is a varying moisture content, the upper portion of the sample is alternately more moist and more dry than the lower portion and with some soils, the addition of water without stirring tends to produce puddling and to form a crust. The second procedure will not prevent loss of water completely and does not permit perfect diffusion of air into the container.
>
> The methods in vogue for studying the process of nitrification contain many errors, which must be largely eliminated before the problem of soil bacteriological differences can be satisfactorily attacked.

Allen and Bonazzi suggested the construction of a constant temperature "humidor" in which nitrification studies can be conducted.

Water movement in the liquid form in soils is practically nil below the field carrying capacity. Evaporation takes place an increment at a time at the surface of a soil mass. When a sample of soil is in a container, the drying surface includes not only the top but the sides and bottom as well. These additional evaporation surfaces result when a wet soil mass shrinks upon drying. The center of the cake will be more moist than the outer edges. Therefore it is very difficult, if not impossible, to maintain uniform distribution of water by weighing containers with soils every two or three days and adding more water as needed. With frequent additions of water, the surface of the sample may become puddled which greatly reduces aeration and influences nitrification materially.

One hole rubber stoppers effectively stop loss of moisture from the soil samples when the containers are incubated in a nearly saturated atmosphere. Furthermore, uniform distribution of moisture is maintained throughout the incubation period which allows better nitrification and greatly reduces the labor involved in conducting the nitrification studies. The exchange of air through the one hole stopper apparently is sufficient for maximum nitrification rates.

The elimination of the necessity of adding water at frequent intervals throughout the period of incubation in nitrification studies permits the use of smaller samples of soil in smaller containers. The smaller samples in smaller containers are essential in a soil testing procedure since space for incubating a large number of samples becomes important. The small bottles fit into trays which permit handling 12 samples at a time in the analytical procedures.

Nitrification takes place over a rather wide range of moisture conditions. There is much conflicting data on the optimum moisture content for nitrification. Considering the techniques employed in wetting soil samples and in maintaining the moisture, the variations in results are understandable. In general, the nitrification rates are not greatly different on medium textured soils in the range from 20 to 30 per cent moisture. Bhaumik and Clark (9) report greatest microbiological activity in soils at the aeration porosity limit, as measured by convention as 50 cm water tension. They obtained their moisture levels by determining the moisture constant for the soil and then adding the amount needed.

Results obtained in this study with tension plates indicate maximum nitrification is obtained at moisture contents approximating aeration porosity. Nitrification rates were greatest at 100 cm water tension on the four samples studied but on two of the samples the rate was equally good at 50 cm tension. On the other two samples the moisture content was too high at 50 cm tension and nitrification was greatly retarded.

For nitrification rate determinations it should not be too difficult to design a cup with a porous bottom which would permit moistening the samples from the bottom and thereby reducing to a minimum changes in physical condition of the soil during application of water. Suction or pressure could be applied equivalent to 100 cm of water. The bottom of the cup could be capped to prevent loss of moisture and the top covered with a one hole rubber stopper to permit sufficient movement of air.

Several modifications were made in the phenoldisulfonic acid procedure for nitrate analysis. These changes greatly speeded up the procedure without sacrificing accuracy. The 2 ml portions of filtrate can be pipetted much more rapidly with a pressure bulb pipette than with a regular volumetric pipette. Evaporation of 2 ml aliquots takes place more quickly than the 10 ml usually removed. In the revised procedure, one person could easily analyze about 12 samples per hour for nitrate analysis.

EVALUATION OF FIELD SAMPLING PROCEDURES FOR NITRIFICATION

Introduction

There are several important steps in soil testing, one of which is obtaining a representative sample from the area that is to be tested. Soil nitrogen that is available for plant growth is released through the decay of organic matter. Although the more recently added undecomposed fraction of organic material is a relatively small percentage of the total soil organic matter, it plays an important role in nitrogen release for plants. If the fresh organic material has a wide carbon/nitrogen ratio, microorganisms will in the decomposition process assimilate nitrogen until the ratio is narrowed.

Since decomposition is greatly influenced by climatic conditions including moisture and temperature, the question arises relative to the effect of season when the soil sample is taken upon nitrification rate. In conjunction with this problem is the question how to take a representative sample for nitrification studies since undecomposed organic matter influences the results.

Historical

Various procedures have been used to take soil samples. Gowda (43) in sampling 36 rotation plots, removed a volume two feet square and seven inches deep from each plot. After thorough mixing, a subsample was taken for laboratory analysis. Post (93) pointed out that the errors in laboratory procedure are low in comparison to field error in sampling. He found that plots which appear fairly uniform may show a large variation in nitrogen content among samples. The application of manure increased soil variability. Cline (21) emphasized the necessity of complete randomization in taking samples for estimates of significance or fiducial limits. Rigney and Reed (98) studied variations in duplicate samples of 20 cores each from each half of 20 regularly shaped fields with pH, organic matter, exchangeable calcium, magnesium, potassium, exchange capacity, and soluble phosphorus but they did not measure nitrogen or nitrification rates.

Boatman (11) tried various methods of handling samples taken in the field so their nitrifying power could be accurately determined in the laboratory. Air drying the samples as quickly as possible gave the best results. Mortenson and Duley (85) found the nitrate content of a fine sandy loam soil became reduced immediately after drying and then gradually increased for a period of several weeks. Landrau (65) obtained a higher nitrification rate on soil samples air dried prior to incubation than on samples maintained at field moisture content. A very good correlation was obtained, however, between the two procedures.

The nitrate content fluctuates greatly in the soil. Several have found accumulation of nitrates in field soils is greatest in late spring and early summer (43, 62, 101) with a decrease in summer months. A slight increase usually occurs again in the fall. Batham and Nigam (6) in India found the greatest accumulation of nitrates during summer months and the least in the winter months. Russell (101) found nitrate fluctuations greater on loams than on sands or clays. King and Whitson (62) showed stirring of soil by cultivation increased nitrate content to a depth of three inches.

Smith, Brown and Millar (104) showed microbiological activity in soil is rhythmical in nature as indicated by evolution of carbon dioxide but were unable to offer a satisfactory explanation. Brown and Gowda (16), Griffith (47), and Cheney (20) found periods of intensive nitrification alternating with periods of lesser action. The length of these periods and the time of their occurance varied with treatment of the soil. Cheney attributed his results to the number and activities of various soil microorganisms present. Griffith

(47) suggested two opposing factors were at work, one favoring nitrate accumulation and the other nitrate destruction, both being quite rapid under suitable conditions.

Many workers have shown that addition of organic matter of various forms greatly influence nitrification and the accumulation of nitrate in soils (2, 7, 12, 31, 59, 76, 77, 78, 88, 91, 117, 118, 121). Materials having a wide carbon/nitrogen ratio depressed nitrate accumulation to a greater extent than materials having a relatively narrow carbon/nitrogen ratio (54, 78, 84). Nitrification rates or nitrate accumulation in soils following growth of legumes is associated with the higher nitrogen content of the legumes in comparison to non-leguminous plants (80, 118, 121). When cellulose material is added with alfalfa or sweetclover tops to widen the carbon/nitrogen ratio, nitrate accumulation is depressed (54). Broadbent and Bartholomew (13) showed the rate of decomposition of oat straw in soil is inversely related to the quantity of straw added. The less rapid rate of decomposition at the higher rate of addition of straw could not be explained on the basis of inadequate aeration or deficiencies of nitrogen and phosphorus.

In studying nitrification of plant residues, Whiting (118) found legumes as a class nitrify distinctly faster than non-legumes. Young green tops nitrify much more rapidly than dry mature plants. He found a high content of easily hydrolyzable nitrogen gives very rapid nitrification if unencumbered with cellulose. In another report, Whiting and Richmond (119) showed the nitrification of biennial sweetclover was dominated in the early stages by roots because they contained 66 per cent of the total nitrogen of the plant and 87 per cent of this was water soluble. Roots of plants in general furnish food for microorganisms which utilize and immobilize nitrogen (79). During early growth corn does not depress nitrate accumulation but does later in the season. Goring and Clark (42) reported mineralization under crops seemed to be less than on fallow soils even when the amount of nitrogen taken up by crops is considered. The extent of nitrogen deficit under cropped versus fallow was correlated to total weight of roots, to the nitrogen content of the crop grown, and to the increase in number of microorganisms that occurs with plant growth.

The decomposition of timothy residues in soils extends over a longer period of time than for clover residues according to the observations of Wilson and Wilson (121). The clover residues are more easily oxidized than residues from timothy and the number of microorganisms rises in a relatively short time to higher figures. Patrick (91) also found that timothy residues depressed nitrification over no treatment. Even clover residues seemed to cause a temporary depression of nitrates. Corn residues had to be added in amounts much greater than found in the field to affect soil nitrates.

McCalla and Russel (82, 83) studied the effect of grain crop and sweetclover residues left on the surface of the soil upon nitrification. In preparation of seedbeds for corn, variations in nitrate production by various cultural practices was apparently related to the rapidity with which soil under the various treatments became warm in the spring. Where residues were left on the surface, the total nitrate content of the soil to a depth of six feet was slightly lower than where it had been incorporated into the soil.

Since crop residues greatly influence nitrification rates, cropping systems followed should also effect nitrification. The physical condition of the soil influences nitrification. Lyon and Bizzell (77) determined the nitrate content of soil under timothy, corn, potatoes, oats, millet, and soybeans and found it different for each crop when growing on the same soil. There was a characteristic relationship between crop and nitrate content of the soil at different growth stages. King and Whitson (62) reported clover and alfalfa appeared to hold the nitrate content in the soils to a lower level than corn, oats, or potatoes but after the crops were removed, nitrification was faster on the legume areas.

Kubota, Rhoades, and Harris (64) determined nitrification rates on soil samples taken from rotation plots of an irrigated Chestnut soil in western Nebraska. Nitrification rate was closely associated with the total nitrogen and oxidizable organic content of the soil. The results showed, however, that applications of manure and alfalfa in the rotation had a greater influence upon nitrification than on total nitrogen or oxidizable organic matter.

A highly significant correlation was obtained between nitrification rate of the soil and yield of corn by Hanway (49) on the rotation plots near Lincoln, Nebraska. The nitrification rates were highest immediately following a legume in the rotation or after application of manure. It decreased with the length of time interval between the sampling and the plowing under of legume or manure. Allison and Sterling (3) studied nitrification rates on soil samples taken from selected field plots on a Cheyenne fine sandy loam soil near Mandan, North Dakota. The nitrate formation varied directly with the total nitrogen content of the soil regardless of past agronomic practices and negatively with the percentage decrease in total nitrogen during the 33-year cropping period. The results indicated that under natural conditions, the more fertile soils are able to supply more nitrates to crops initially and also over a longer growing season. Humfeld and Erdman (53) found manure and lime caused a great increase in nitrification rates on the rotation plots at Ames, Iowa. Manure alone had no effect. In general there was a good correlation between treatment and nitrification rates in the rotation plots.

Samples were taken from 99 plots at substations in western Kansas by Gainey, Sewell, and Latshaw (~~38~~ 41) in 1916, 1927, and again in 1928. The principal factor governing nitrogen balance and nitrification rates seemed to be the nitrogen content of the soils at the beginning of the period.

Landrau (65) studied the influence of cropping and cultural practices upon the seasonal trend in nitrification rates of soils growing corn. Soil samples were taken from the corn plots at planting time, when the corn was 18 inches tall, 68 inches tall, 104 inches tall, and at maturity. He obtained a significant difference in nitrification rate of soils taken at the different times in relation to the cropping system and cultural practices. Nitrification rates were highest at the first or second sampling times and decreased gradually thereafter. This decrease was found in second-year corn as well as corn after a legume. On one test the decrease was even greater on continuous corn than on corn following clover or on second-year corn that had been manured.

Experimental

The first- and second-year corn plots in two rotations at the Agronomy Farm near Ames and the Page County rotation plots near Clarinda were selected for the evaluation of field sampling procedures for nitrification. The two rotations were corn-corn-oats with sweetclover for green manure, and corn-corn-oats-meadow. The meadow was a mixture of alfalfa, red clover and timothy.

The rotation plots at the Agronomy Farm at Ames were started in 1942 on Webster silt loam soil. All crops in the various rotations are grown each year and each treatment replicated two or three times. The plots are 21 feet wide and 30 feet long with seven foot borders. All of the plots were plowed on May 16 for the 1951 corn crop and the corn was surface planted a week later.

The rotation plots at Clarinda on Marshall silt loam soil were started in 1943. The treatments are in triplicate and all crops appear each year. The plots are 20 feet wide and 47 feet long. They were fall-plowed and the corn planted with a lister on May 24. All plots received 100 pounds per acre of 0-20-10 fertilizer per year.

The plots were sampled the first time in May about one week after the corn was planted. The second sampling was in

July when the corn was 30 to 40 inches tall. The third and last sampling was in the middle of September. At each sampling date, three composite samples were taken from each plot. A composite sample consisted of 10 cores taken with a soil tube to a depth of six inches. The cores were taken at random over each plot. In the first sampling of the Clarinda plots, the cores were taken from the sides of the lister furrow or at ground level. At all sampling dates the soils were moist almost to field carrying capacity.

The composite samples were placed in large paper bags and taken to the laboratory where they were crumbled and mixed by hand so no clods remained that were larger than one-fourth to three-eighths inches in size. They were spread on paper towels in 8 x 8 inch tin pans and by use of fans air was forced over them. Within 18 hours the samples were air dried to a moisture content less than five per cent. They were then crushed with a steel rolling pin to pass a 10-mesh screen and thoroughly mixed.

Nitrification rate determinations were made using 25 grams of soil in pint milk bottles. The samples were moistened with 6.25 ml of distilled water. The bottles were closed with one hole rubber stoppers and the samples incubated at 35° C for three weeks in a nearly saturated atmosphere. Nitrate nitrogen was determined by the modified phenoldisulfonic acid method that has been described previously.

As an aid in interpreting variability among sampling data and plots, pH, available phosphorus, and available potassium were determined by procedures used in the Iowa soil testing laboratory on one composite sample from each plot for each of the three sampling periods.

Plant residues were collected in the spring from material plowed under on the rotation plots at the Agronomy Farm near Ames. The residues were taken from the first-year corn plots on both rotations. Material was collected also from the second-year corn on the corn-corn-oats-meadow rotation. The organic residues were placed in crocks of distilled water for an hour to loosen adhering soil. Then they were rinsed with distilled water and dried at 65° C. After drying, the material was finely ground in a Wiley mill.

The following results were obtained in an analysis of the residue.

Residue from:	% Nitrogen	% Phosphorus	% Potassium
Meadow	1.64	0.15	0.51
Clover	1.83	0.22	0.87
Corn	0.41	0.08	0.20

Soil samples taken in May from first-year corn plots of both rotations and second-year corn from the corn-corn-oats-meadow rotations were incubated with the addition of residue from the same plots. For the incubation study, 25 grams of soil and 25 and 50 milligrams of residue were placed in pint milk bottles. Then 6.25 ml of distilled water were added and the samples incubated in the same manner as the other samples.

Fields five to eight acres in size were selected by the soil testing laboratory in Fayette, Buchanan, Monona and O'Brien counties to determine seasonal variation in pH and available phosphorus and potassium. Several samples were taken during 1949 and 1950 from these fields. Each sample was a composite of 10 to 15 cores that were taken with soil sampling tubes. The samples were air dried, crushed to pass a 10-mesh screen and thoroughly mixed. In order to gain more information about nitrification in relation to time of sampling, nitrification rates were also determined on these samples. For this study, 10 grams of soil were placed in 30 ml bottles moistened with 2.5 ml of distilled water and incubated as previously described.

Results

The results of the study on sampling procedure are shown in Tables 14 to 24, inclusive.

The variability in duplicate determinations of the same sample was quite small. It was less than 1.0 ppm for the initial nitrate and not more than 3.0 ppm and usually less than 1.0 ppm for nitrification rate.

Table 14. The nitrification rate of soils from first- and second-year corn in an oats, meadow, corn, corn rotation at the Agronomy Farm near Ames, in relation to time of sampling

Plot and sample no.	Initial nitrate, ppm			Nitrification rate, ppm			Yield of corn, 1951
	May	July	Sept.	May	July	Sept.	Bu./acre
			First-year corn				
7B-1	4.4	11.0	4.0	64.8	53.0	45.2	88.8
7B-1	4.6	11.4	4.0	64.6	52.6	47.2	--
7B-2	4.0	9.2	4.4	64.4	56.8	49.2	--
7B-2	4.2	8.6	4.4	65.0	56.2	49.2	--
7B-3	4.4	8.4	4.4	64.8	55.6	48.0	--
7B-3	4.2	8.2	4.2	64.2	54.6	46.6	--
7E-1	4.2	5.6	3.8	59.8	47.2	38.6	93.5
7E-1	4.4	5.2	3.6	58.4	47.2	40.0	--
7E-2	4.0	5.2	4.0	57.4	50.4	38.8	--
7E-2	4.0	5.6	4.0	57.0	50.0	38.0	---
7E-3	4.0	6.2	4.0	58.4	47.8	39.6	--
7E-3	3.8	6.6	4.2	59.8	47.4	39.4	--
			Second-year corn				
6A-1	3.0	3.6	2.6	45.0	47.2	39.4	47.1
6A-1	3.2	3.6	2.2	44.8	45.2	38.6	--
6A-2	3.6	3.4	2.4	45.6	45.4	34.0	--
6A-2	3.8	2.8	2.4	44.2	47.2	33.6	--
6A-3	3.8	3.2	2.2	44.2	46.8	35.8	--
6A-3	3.8	3.0	2.8	44.6	47.8	36.0	--
6C-1	4.0	3.2	2.6	39.2	38.0	37.4	73.3
6C-1	4.2	3.4	2.8	39.8	39.4	37.6	--
6C-2	4.2	3.4	2.8	40.2	39.4	37.2	--
6C-2	4.0	3.4	2.8	40.4	40.6	36.8	--
6C-3	4.4	3.6	2.8	41.2	40.4	34.0	--
6C-3	4.0	3.6	2.8	40.8	38.4	36.4	--

Table 15. Analysis of variance table

Source	d.f.	May		July		September	
		S.S.	M.S.	S.S.	M.S.	S.S.	M.S.
				Initial nitrate			
Replicates	1	.202	.202	19.082	19.082	0.002	0.002
Treatments	1	.735	.735	108.375	108.375	13.202	13.202
Experimental error	1	1.042	1.042	22.815	22.815	0.602	0.602
Among composites	8	0.920	0.115	11.133	1.392	0.373	0.047
Among determinations	12	.260	0.022	0.740	0.062	0.340	0.028
Total	23	3.158		162.145		14.518	
				Nitrification rate			
Replicates	1	169.60	169.6	281.535	281.535	100.042	100.042
Treatments	1	2177.41	2177.4	442.042	442.042	287.042	287.042
Experimental error	1	4.342	4.342	0.882	0.882	117.042	117.042
Among composites	8	7.453	0.932	27.747	3.468	43.813	5.477
Among determinations	12	3.780	0.315	8.740	0.728	7.70	0.642
Total	23	2362.585		760.945		555.64	

Table 16. The nitrification rate of soils from first-year corn in an oats, clover, corn, corn rotation at the Agronomy Farm near Ames in relation to time of sampling

Plot and sample no.	Initial nitrate, ppm			Nitrification rate, ppm			Yield of corn, 1951
	May	July	Sept.	May	July	Sept.	Bu./acre
			First-year corn				
3A-1	2.6	5.0	2.6	42.6	37.8	38.6	62.8
3A-1	3.0	5.0	2.6	42.6	37.8	38.2	--
3A-2	3.0	4.8	2.6	44.2	37.6	35.4	--
3A-2	2.8	5.0	2.8	45.2	37.8	36.8	--
3A-3	2.8	4.6	3.0	45.6	37.4	35.0	--
3A-3	3.0	4.2	2.8	44.6	37.8	32.8	--
3C-1	2.8	5.8	2.8	41.6	34.8	34.0	73.6
3C-1	3.0	5.6	2.8	41.0	34.6	35.2	--
3C-2	3.0	4.9	2.6	40.6	35.5	33.4	--
3C-2	2.8	5.6	2.8	40.4	35.6	33.6	--
3C-3	3.0	6.2	2.6	41.4	34.6	35.8	--
3C-3	3.0	6.0	3.0	41.4	35.2	36.2	--
3D-1	3.0	3.6	2.4	42.2	40.4	36.4	50.7
3D-1	2.8	3.6	2.2	42.4	42.4	37.0	--
3D-2	3.0	3.8	2.4	42.4	41.8	40.8	--
3D-2	3.0	4.0	2.4	42.8	42.0	40.8	--
3D-3	3.0	3.8	2.8	42.6	41.0	36.8	--
3D-3	3.0	3.8	3.0	42.6	41.4	36.6	--

Table 17. The nitrification rate of soils from second-year corn in an oats, clover, corn, corn rotation at the Agronomy Farm near Ames in relation to time of sampling

Plot and sample no.	Initial nitrate, ppm			Nitrification rate, ppm			Yield of corn, 1951
	May	July	Sept.	May	July	Sept.	Bu./acre
				Second-year corn			
4A-1	4.0	3.8	2.6	37.2	37.0	37.8	53.6
4A-1	3.8	4.0	2.6	37.4	35.6	36.6	--
4A-2	4.0	4.6	3.0	37.2	36.2	33.8	--
4A-2	4.0	4.6	2.6	37.6	36.2	34.2	--
4A-3	4.2	3.8	2.8	37.4	34.6	34.8	--
4A-3	4.2	3.6	3.2	36.6	36.0	34.8	--
4C-1	4.2	3.6	3.0	38.2	39.6	33.8	55.3
4C-1	4.2	3.6	3.0	38.2	40.0	35.4	--
4C-2	4.0	3.6	3.2	40.4	38.4	33.6	--
4C-2	4.0	3.8	2.8	39.2	38.2	33.2	--
4C-3	4.0	3.4	2.8	40.0	40.2	33.6	--
4C-3	3.8	3.8	2.8	41.0	39.0	35.6	--
4D-1	3.8	3.6	3.0	37.4	33.2	28.6	49.8
4D-1	4.0	3.6	2.8	36.8	35.2	29.2	--
4D-2	4.0	3.6	2.8	37.6	30.0	31.2	--
4D-2	4.0	3.8	3.0	36.8	27.0	29.4	--
4D-3	4.0	3.8	2.6	38.8	30.6	28.6	--
4D-3	4.0	3.6	3.0	38.8	33.2	29.4	--

Table 18. Analysis of variance table

Source	d.f.	May		July		September	
		S.S.	M.S.	S.S.	M.S.	S.S.	M.S.
				Initial nitrate			
Replication	2	0.0067	0.0034	5.74	2.87	0.136	0.068
Treatments	1	0.0424	0.0212	8.12	8.12	0.321	0.321
Experimental error	2	10.67	10.67	5.985	2.99	0.109	0.055
Among composite	12	0.24	0.02	2.15	0.18	0.747	0.06
Among determinations	18	0.24	0.013	0.585	0.03	0.540	0.030
Total	35	11.20		22.58		1.852	
				Nitrification rate			
Replications	2	2.136	1.07	2.35	1.17	24.64	12.32
Treatments	1	176.00	176.00	57.00	57.00	99.33	99.33
Experimental error	2	43.31	21.65	302.87	151.43	128.67	64.33
Among composites	12	17.68	1.47	38.11	3.18	64.40	5.37
Among determinations	18	3.44	0.19	15.07	0.84	10.78	0.60
Total	35	242.57		415.39		327.83	

Table 19. The nitrification rate of soils from first-year corn in an oats, meadow, corn, corn, rotation at Clarinda Experimental Farm in relation to time of sampling

Plot and sample no.	Initial nitrate, ppm			Nitrification rate, ppm			Yield of corn, 1951
	May	July	Sept.	May	July	Sept.	Bu./acre
				First-year corn			
16-1	12.2	7.8	3.0	51.8	64.0	59.0	91.7
16-1	12.0	8.0	3.4	50.8	59.8	56.2	--
16-2	10.2	7.2	3.4	51.4	56.8	51.8	--
16-2	10.0	6.6	3.4	52.8	57.4	52.2	--
16-3	12.4	6.6	3.4	48.8	58.2	51.8	--
16-3	12.2	7.0	3.4	51.8	58.6	53.0	--
63-1	11.2	5.2	3.4	46.0	51.6	53.4	103.5
63-1	10.8	4.8	3.6	47.2	52.8	51.2	--
63-2	10.0	6.0	3.4	48.0	52.8	51.0	--
63-2	9.4	5.8	3.4	49.0	53.0	52.6	--
63-3	11.2	5.8	3.8	45.2	51.0	51.8	--
63-3	11.2	5.8	3.8	44.0	52.2	51.8	--
96-1	12.8	6.6	3.2	51.6	52.2	58.4	85.6
96-1	12.8	6.6	3.0	51.6	53.4	59.8	--
96-2	12.4	6.0	3.0	53.2	52.8	54.2	--
96-2	12.2	6.2	3.0	54.2	52.2	53.8	--
96-3	12.2	6.0	3.4	53.8	54.0	57.8	--
96-3	12.6	5.8	3.2	52.2	54.6	60.8	--

Table 20. The nitrification rate of soils from second-year corn in an oats, meadow, corn, corn rotation at Clarinda Experimental Farm in relation to time of sampling

Plot and sample no.	Initial nitrate, ppm			Nitrification rate, ppm			Yield of corn, 1951 Bu./acre
	May	July	Sept.	May	July	Sept.	
				Second-year corn			
20-1	6.6	5.6	2.2	50.2	52.8	50.2	55.9
20-1	7.0	6.0	2.2	47.0	51.2	50.4	--
20-2	6.4	5.8	2.6	42.8	51.4	48.2	--
20-2	7.0	5.4	3.0	44.2	53.4	49.4	--
20-3	7.4	5.2	2.2	43.4	52.8	48.6	--
20-3	7.8	5.0	2.4	41.8	52.2	46.4	--
61-1	5.4	3.8	2.2	44.2	41.8	42.4	66.6
61-1	5.6	4.0	2.2	44.0	42.0	44.4	--
61-2	5.8	3.8	2.2	40.6	40.6	39.8	--
61-2	5.6	3.8	2.2	39.6	40.2	42.6	--
61-3	5.6	4.0	2.2	44.4	45.2	41.4	--
61-3	5.6	3.8	2.2	45.2	45.8	41.8	--
91-1	6.8	3.6	2.2	41.2	51.6	47.0	58.9
91-1	6.8	3.4	2.6	41.2	52.2	46.6	--
91-2	6.0	4.0	2.8	41.6	50.4	42.0	--
91-2	5.8	3.8	2.2	43.8	50.2	43.0	--
91-3	6.4	3.4	2.2	41.6	51.0	47.4	--
91-3	6.0	3.4	2.2	40.4	51.8	48.2	--

Table 21. Analysis of variance table

Source	d.f.	May S.S.	May M.S.	July S.S.	July M.S.	September S.S.	September M.S.
				Initial nitrate			
Replications	2	11.949	5.974	19.216	9.608	0.142	0.071
Treatments	1	246.490	246.490	36.000	36.000	9.201	9.201
Experimental error	2	4.680	2.340	1.620	0.810	0.595	0.298
Among composites	12	10.713	0.893	3.787	0.316	0.827	0.069
Among determinations	18	0.90	0.050	0.680	0.038	0.500	0.028
Total	35	274.732		61.302		11.266	
				Nitrification rate			
Replications	2	69.487	34.743	416.549	208.274	161.536	80.768
Treatments	1	442.401	442.401	341.018	341.018	718.240	718.240
Experimental error	2	87.949	43.974	89.469	44.734	70.207	35.103
Among composites	12	94.093	7.841	60.507	5.042	120.533	10.044
Among determinations	18	21.060	1.170	15.880	0.882	24.04	1.336
Total	35	714.990		923.422		1094.556	

Table 22. The nitrification rate of soils from first-year corn in an oats, clover, corn, corn rotation at Clarinda Experimental Farm in relation to time of sampling

Plot and sample no.	Initial nitrate, ppm			Nitrification rate, ppm			Yield of corn, 1951 Bu./acre
	May	July	Sept.	May	July	Sept.	
First-year corn							
28-1	11.4	8.0	3.4	45.4	48.4	40.2	77.2
28-1	10.8	7.8	3.2	43.2	50.2	40.8	--
28-2	13.2	7.6	3.2	49.2	50.4	44.4	--
28-2	12.8	8.0	3.2	48.8	50.4	44.8	--
28-3	11.8	9.2	3.0	47.0	50.4	40.6	--
28-3	13.2	8.8	3.4	44.0	53.2	43.4	--
33-1	13.2	7.8	3.6	54.8	53.4	48.8	85.7
33-1	13.6	7.4	3.4	52.4	54.6	52.6	--
33-2	11.2	10.0	3.6	46.8	54.0	47.6	--
33-2	10.6	10.0	3.6	47.4	53.6	46.4	--
33-3	13.6	9.2	3.4	48.0	52.4	47.8	--
33-3	14.0	8.8	3.4	47.6	54.0	49.4	--
93-1	6.6	6.4	3.4	41.0	46.4	35.4	77.4
93-1	6.6	5.6	3.0	40.2	47.6	35.8	--
93-2	10.8	5.2	3.4	43.4	45.6	36.2	--
93-2	10.0	5.2	3.4	44.8	45.6	35.8	--
93-3	10.8	5.2	3.4	40.8	43.2	36.2	--
93-3	10.8	5.4	3.4	42.0	42.6	36.2	--

Table 23. The nitrification rate of soils from second-year corn in an oats, clover, corn, corn rotation at Clarinda Experimental Farm in relation to time of sampling

Plot and sample no.	Initial nitrate ppm			Nitrification rate, ppm			Yield of corn, 1951
	May	July	Sept.	May	July	Sept.	Bu./acre
				Second-year corn			
31-1	7.8	5.2	3.0	39.8	46.0	42.2	52.0
31-1	7.8	5.2	3.0	41.8	46.8	41.0	--
31-2	7.8	5.4	2.8	41.0	47.4	39.2	--
31-2	7.4	5.2	2.8	41.4	49.2	38.0	--
31-3	7.0	5.0	2.4	45.0	47.0	40.6	--
31-3	7.0	5.0	2.6	42.2	48.2	38.0	--
53-1	4.0	3.6	3.4	47.6	50.0	38.8	48.5
53-1	4.4	3.6	3.2	44.8	49.6	36.6	--
53-2	3.8	3.6	2.6	46.2	51.6	39.2	--
53-2	4.0	3.4	2.6	44.0	53.0	36.8	--
53-3	4.4	3.4	2.6	44.0	52.6	50.2	--
53-3	4.4	3.4	3.0	42.8	53.8	49.8	--
74-1	5.2	3.4	2.8	44.8	49.8	39.6	45.7
74-1	5.6	3.4	2.6	41.6	47.8	39.4	--
74-2	5.6	4.0	2.6	44.4	48.8	41.2	--
74-2	5.8	3.8	2.6	44.6	47.8	38.0	--
74-3	5.8	3.8	2.6	42.6	47.0	38.6	--
74-3	5.6	4.0	2.8	42.8	48.8	38.0	--

Table 24. Analysis of variance table

Source	d.f.	May		July		September	
		S.S.	M.S.	S.S.	M.S.	S.S.	M.S.
				Initial nitrate			
Replications	2	34.880	17.440	28.247	14.123	0.320	0.160
Treatments	1	286.738	286.738	104.040	104.040	3.004	3.004
Experimental error	2	39.262	19.631	19.940	9.970	0.062	0.031
Among composites	12	36.320	3.027	8.813	0.734	0.893	0.074
Among determinations	18	2.200	0.122	0.760	0.042	0.360	0.020
Total	35	399.400		161.80		4.640	
				Nitrification rate			
Replications	2	125.416	62.708	218.309	109.154	365.929	182.964
Treatments	1	57.254	57.254	3.240	3.240	38.440	38.440
Experimental error	2	70.469	35.234	65.887	32.943	52.987	26.494
Among composites	12	108.813	9.068	41.267	3.439	240.227	20.019
Among determinations	18	30.380	1.688	17.080	0.949	29.080	1.616
Total	35	392.332		345.782		826.662	

The variability in the initial nitrate content between composite samples of 10 cores was not large. Seldom was there more than 2.0 ppm difference and usually it was less than 1.0 ppm. In general, the variability was greatest in the May and July samplings and least in September. The initial nitrate content of the Clarinda plots was higher in May than at Ames, but little difference was found in subsequent samplings. This was due, no doubt, to fall plowing at Clarinda and spring plowing at Ames. In all of the samplings there was less variability generally in the initial nitrate on the Ames plots than at Clarinda.

Among composites from the same plots there was more variation in nitrate production than in initial nitrate. The variation in nitrification among composite samples from the same plot was generally less than 4 ppm. There appeared to be less variability in composite samples taken in July than among composite samples taken in either May or September.

Considerable variability was found between the nitrification rates of replicated plots, especially on the Agronomy Farm near Ames. On the first-year corn following meadow, the nitrate production on plot 7E was 6 to 8 ppm less than on plot 7B. Furthermore, plot 6A was higher than plot 6C. The variation in the initial nitrate between replicated plots was not as great as with nitrification rates.

The trends in the initial nitrate content of the soil samples and in nitrate production at the three sampling periods are illustrated in Figures 8 and 9. The solid lines are first-year corn and the dotted lines second-year corn in the same rotation. Each line represents the average of the duplicate determinations on the three composite samples taken from the respective plots.

At Ames, the initial nitrate content for both rotations was highest in July on the first-year corn plots. On the second-year corn plots the initial nitrate was highest in May and progressively decreased to the September sampling. On the Clarinda plots, the initial nitrate was highest at the May sampling on all plots including first- and second-year corn in both rotations.

At Clarinda the initial nitrate was higher on first-year corn than on second-year corn on both rotations but at Ames the differences were not so pronounced.

The nitrification rates on rotation plots at Ames were highest on samples taken in May and were lowest on samples taken in September. In the May sampling, the nitrate production on first-year corn following meadow was significantly higher than on second-year corn. This difference decreased at subsequent sampling dates. First-year corn after clover

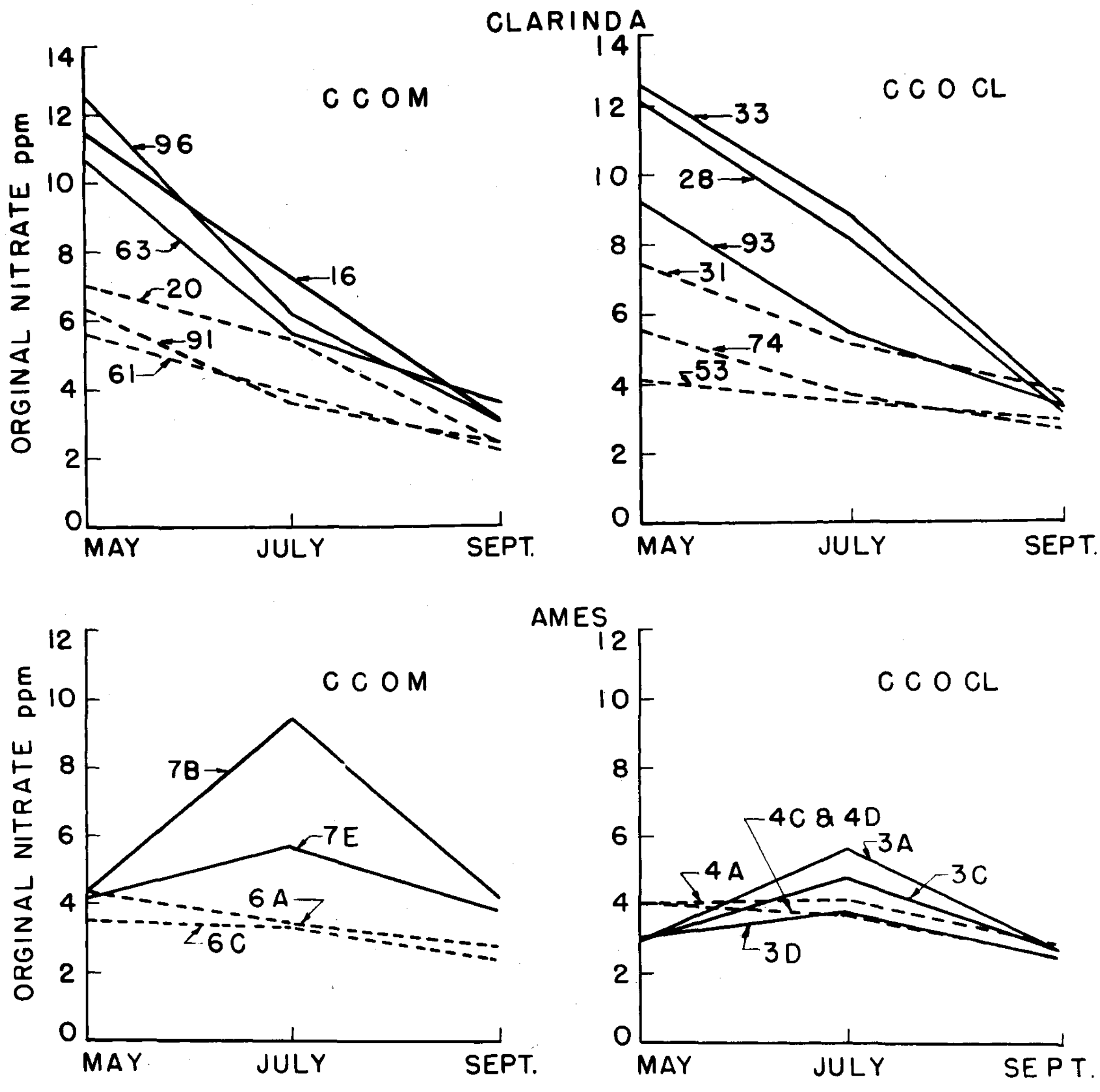

Figure 8. The original nitrate content of soils taken from first- and second-year corn plots of two rotations at Ames and Clarinda in May, July, and September

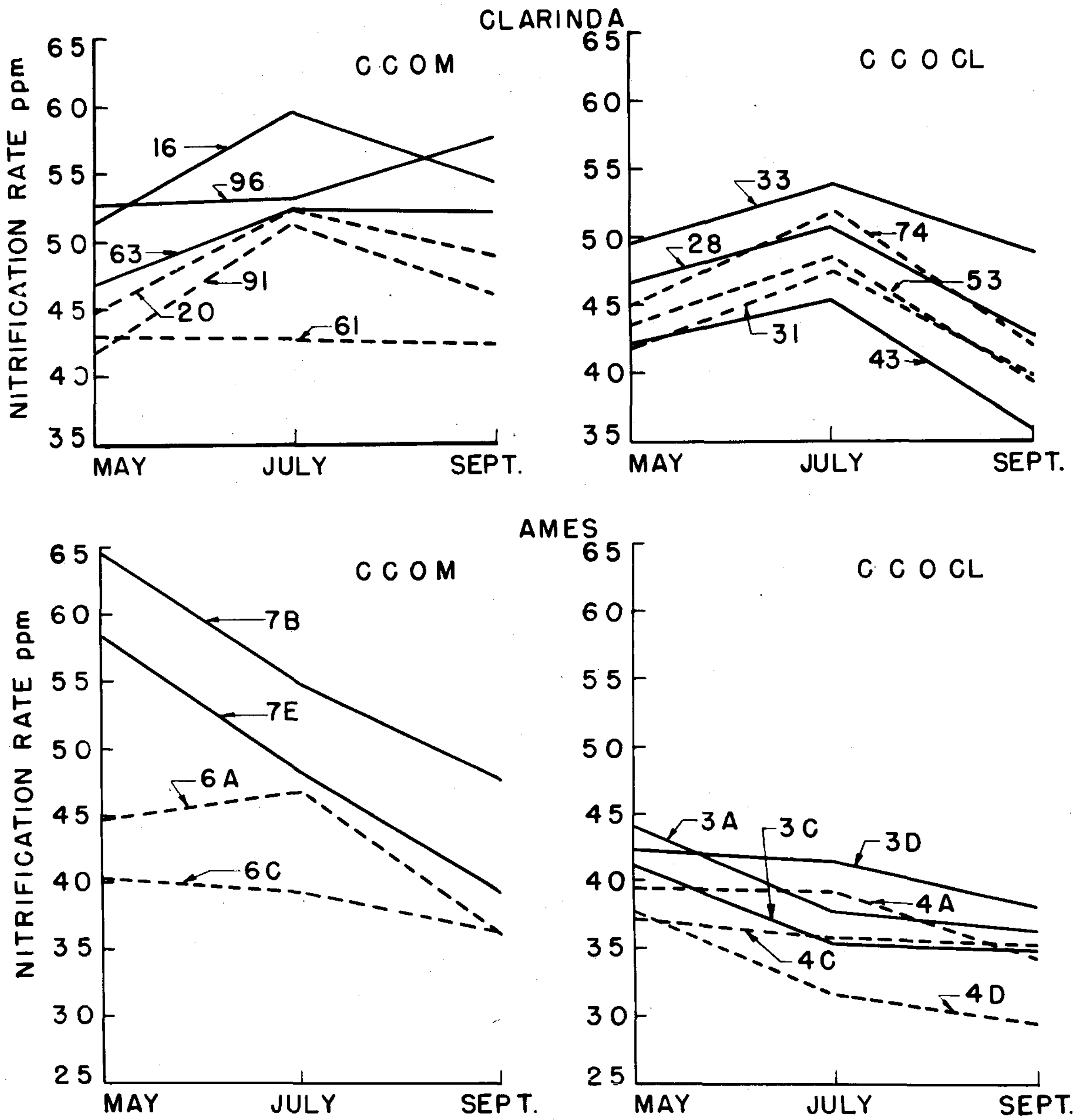

Figure 9. The nitrification rate of soils taken from first- and second-year corn plots of two rotations at Ames and Clarinda in May, July, and September

was a little higher in nitrification than second-year corn but the difference was not as large as following meadow.

The variability between replicate plots cannot be fully explained by pH, available phosphorus, or available potassium measurements (Table 25). In the Clarinda rotations, plot 33 is higher in both initial nitrate and nitrification than plots 28 and 93 which are the same treatment. This might be due to some extent, at least, to the higher pH of plot 33. In the Ames' rotations, plot 4D is lower in nitrification than plots 4A and 4C and it is also the most acid. The difference in pH on the other plots presumably is not enough to influence nitrate accumulation. Both the available phosphorus and potassium tend to reach a maximum in the July sampling. This probably is due to decomposition of organic matter but does not appear to be directly correlated with nitrification. The available phosphorus and potassium at Ames is lower on plot 6C than plot 6A and nitrification is also lower. The same is true of plot 3C in relation to plots 3A and 3D.

The addition of crop residue at one and two tons per acre reduced the nitrification rate in a three-week incubation period as shown in Table 26. The two ton per acre application reduced nitrification more than the one ton application on all soils. The two-ton meadow residue reduced nitrification about 5 ppm compared to 10 ppm for clover residue and 20 ppm for corn residue.

Table 25. The variability of pH, available phosphorus and available potassium in soils in relation to time the samples were taken

Plot number	pH			Available phosphorus pounds per acre			Available potassium pounds per acre		
	May	July	Sept.	May	July	Sept.	May	July	Sept.
				Ames rotation plots					
3A	6.4	6.2	6.3	7.5	17.5	8.0	176	192	168
3C	6.5	6.4	6.5	4.0	9.0	5.5	172	180	168
3D	6.0	6.0	6.1	7.5	23.0	15.0	184	224	208
4A	6.3	6.4	6.3	7.5	10.0	9.0	164	196	172
4C	6.2	6.3	6.3	8.0	17.5	9.0	184	196	188
4D	5.9	6.0	6.0	7.5	8.5	11.0	160	156	156
7B	6.1	6.1	6.1	8.5	12.0	8.5	168	172	172
7E	6.0	6.0	5.9	5.5	17.5	7.5	128	152	140
6A	6.0	6.2	6.1	7.5	10.5	17.0	208	240	248
6C	6.4	6.4	6.3	3.0	7.5	4.5	140	212	172
				Clarinda rotation plots					
16	6.2	6.4	6.5	21.0	20.0	11.0	>400	>400	>400
63	6.3	6.5	6.6	13.0	17.5	17.5	>400	>400	>400
96	6.4	6.5	6.6	17.0	17.5	16.0	>400	>400	>400
20	6.5	6.6	6.8	15.0	20.0	14.0	>400	>400	>400
61	6.4	6.5	6.4	19.0	17.5	17.5	>400	>400	>400
91	6.5	6.6	6.7	17.0	21.0	14.0	>400	>400	>400

Table 25 (Continued)

Plot number	pH			Available phosphorus pounds per acre			Available potassium pounds per acre		
	May	July	Sept.	May	July	Sept.	May	July	Sept.
28	6.7	6.7	6.8	19.0	15.0	15.0	>400	>400	>400
33	7.2	7.2	7.2	28.0	33.0	20.0	>400	>400	>400
93	6.4	6.4	6.5	16.0	22.0	12.0	>400	>400	>400
31	6.6	6.7	6.8	16.0	23.0	13.0	>400	>400	>400
53	6.2	6.4	6.5	15.0	17.0	16.0	>400	>400	>400
74	6.4	6.4	6.5	15.0	13.0	11.0	>400	>400	>400

Table 26. The effect of adding one and two tons per acre of crop residue upon nitrification

Plot Number	Treatment	Nitrate nitrogen production - ppm		
		no residue	1 T/A residue	2 T/A residue
3A-1	Clover residue	66.8	57.0	57.4
3A-1		66.0	59.4	55.0
3D-1		67.2	58.4	57.2
3D-1		67.2	57.6	56.8
7E-1	Meadow residue	76.4	70.0	71.6
7E-1		74.0	72.4	70.4
7B-1		87.2	86.2	81.6
7B-1		90.0	84.6	83.6
6A-1	Corn residue	58.8	46.9	40.0
6A-1		57.2	49.5	39.6
6C-1		59.2	42.4	39.6
6C-1		56.4	45.2	36.4

A variation in nitrification rates may occur from one year to another as indicated in Tables 27 and 28. The nitrification rates on all of the fields were higher in 1950 than in 1949 regardless of crops being grown. During either 1949 or 1950 the variability in nitrification rates or the initial nitrate content was not large. The initial nitrate in the corn fields of Fayette county was higher on the dates when samples were taken in 1949 than on the same fields a year later when planted to oats. There was little difference in

Table 27. The influence of time of year soil samples are taken in relation to crop grown upon nitrification rates in Fayette and Buchanan counties in 1949 and 1950

Sample	Month	Crop	Original NO_3	Nitrification rate
		Fayette county		
R462	Oct. 1949	Corn	4.0	46.4
R472	Nov. 1949	Corn	7.5	48.6
A449	June 1950	Oats	2.8	57.7
A478	July 1950	Oats	3.0	58.6
A1179	Sept. 1950	Oats	2.9	58.7
R260	June 1949	Corn	7.25	64.8
R463	Oct. 1949	Corn	7.0	61.7
R473	Nov. 1949	Corn	11.4	61.2
A450	June 1950	Oats	2.8	71.5
A479	July 1950	Oats	2.9	71.9
A1180	Sept. 1950	Oats	3.3	69.3
		Buchanan county		
R263	June 1949	Oats	2.3	51.9
R461	Oct. 1949	Oats	2.2	52.3
R471	Nov. 1949	Oats	2.5	54.7
A452	June 1950	Corn	2.5	58.0
A477	July 1950	Corn	2.9	52.1
A1178	Sept. 1950	Corn	3.1	58.5

Table 28. The influence of time of year soil samples were taken in relation to crop grown upon nitrification rate in Monona county in 1949 and 1950

Sample	Month	Crop	Original NO_3	Nitrification rate
R279	Aug. 1949	Corn	2.2	23.0
R302	Sept. 1949	Corn	3.0	28.1
A351	May 1950	Oats-Alf. & Br.	5.6	42.0
A446	June 1950	Oats-Alf. & Br.	2.6	41.6
A480	July 1950	Oats - Alf. & Br.	2.6	41.4
A930	Sept. 1950	Oats-Alf. & Br.	2.5	41.9
R280	Aug. 1949	Corn	2.7	23.8
R303	Sept. 1949	Corn	3.0	24.2
A352	May 1950	Oats-Alf. & Br.	5.6	39.5
A448	June 1950	Oats-Alf. & Br.	3.0	51.0
A481	July 1950	Oats-Alf. & Br.	2.6	52.2
A931	Sept. 1950	Oats-Alf. & Br.	3.4	38.4
R281	Aug. 1949	Corn	2.3	21.7
R304	Sept. 1949	Corn	2.6	28.7
A353	May 1950	Oats-Alf. & Br.	5.6	44.1
A447	June 1950	Oats-Alf. & Br.	4.2	44.2
A482	July 1950	Oats-Alf. & Br.	2.7	37.4
A929	Sept. 1950	Oats-Alf. & Br.	3.5	41.9

the initial nitrate content of the other fields from one year to the next.

The variability in nitrate production in samples taken at monthly intervals from four fields in O'Brien county during the summer of 1950 was less than 10 ppm as shown in Table 29. In the two corn fields the initial nitrate decreased in August and September as compared to June and July but the nitrification rate increased. There was little difference in the initial nitrate content of the oat and red clover fields during the four-month period but the nitrification rate decreased slightly in August and September as compared to June and July.

Discussion

The nitrification of soil samples taken from first-year corn after meadow plots at Ames and Clarinda was markedly greater than second-year corn plots in the same rotation. First-year corn following sweetclover was higher in nitrification also than second-year corn, but the difference was not as pronounced as on the meadow plots. The differences were larger in May and July than in September. These results are similar to those reported by Landrau (65) on nitrification studies of rotation plots near Lincoln and Holdrege, Nebraska. Higher nitrification rates and a greater difference in nitrate

Table 29. The influence of time of year soil samples were taken in relation to crop grown upon nitrification rate in O'Brien county in 1950

Sample	Month	Crop	Original NO_3	Nitrification rate
A376	June	Corn	6.4	43.4
A472	July	Corn	10.7	44.1
A889	Aug.	Corn	4.6	50.4
A1113	Sept.	Corn	4.1	53.1
A377	June	Corn	9.5	44.2
A473	July	Corn	10.8	42.7
A890	Aug.	Corn	3.7	45.9
A1114	Sept.	Corn	4.1	46.5
A378	June	Oats & Red clover	3.6	47.9
A474	July	Oats & Red clover	2.8	46.8
A891	Aug.	Oats & Red clover	3.7	39.1
A1115	Sept.	Oats & Red clover	4.1	40.7
A379	June	Oats & Red clover	3.7	42.5
A475	July	Oats & Red clover	2.3	37.6
A892	Aug.	Oats & Red clover	3.7	37.3
A1116	Sept.	Oats & Red clover	2.6	37.3

production between first- and second-year corn was found on the Iowa soils than Landrau found with the Nebraska soils. This may have been due, in part, to differences in the nitrification procedures followed.

Yields of corn as well as nitrate production during 1951 on the first-year corn plots were higher in the meadow rotation than in the clover rotation at both Ames and Clarinda. The addition of two tons per acre of residue from the meadow plots reduced the nitrification about 5 ppm compared to a reduction of 10 ppm by the addition of residue from the clover plots. An analysis of plant residues indicate a slightly higher nitrogen, phosphorus, and potassium content for the clover residue compared to the meadow residue so this difference cannot be attributed to the carbon/nitrogen ratio. The meadow plots are higher in fertility as reflected in both corn yields and nitrate production.

A difference was found to exist in the nitrate production and the initial nitrate content of soils in relation to the time of the season in which the samples were taken. The lowest nitrification rate and initial nitrate content was found in the September samplings on almost all of the plots. The nitrate production was highest in May on the Ames plots and highest in July on the Clarinda plots. The reverse situation was true for the initial nitrate. It was highest in July at

Ames and highest in May at Clarinda. The amount of variability, which was less than 10 ppm on almost all of the plots, was almost as large in the initial nitrate content as in nitrate production.

The variability of nitrate production between duplicate determinations was less than the variability among composite samples of the same plot. The variability among composite samples, however, was much less than among replicated plots of the same treatment. This was especially true of the rotation plots at Ames. The differences in replicated plots were also evident in pH, and available phosphorus and potassium. Some of the plots that were lower in available phosphorus and potassium were lower in nitrification. Nitrate production was also related to acidity in some instances. However, not all of the differences among replicated plots could be accounted for in this way and no doubt other important factors such as organic matter content had a marked influence on nitrate production.

The variabilities among replicated plots make it difficult to show differences in relation to treatments or time of year of sampling. The difference was less than 20 ppm in all instances and generally it was less than 10 ppm. For a general evaluation of available nitrogen in the soil, the results indicate samples may be taken any time of the year with no greater variability than is obtained in phosphorus or potassium tests.

THE RELATIONSHIP BETWEEN NITRIFICATION, pH, AND AVAILABLE PHOSPHORUS AND POTASSIUM

Introduction

In the interpretation of soil test results for predicting response from application of fertilizer, factors directly or indirectly effecting release of the element for plant growth must be considered. Since recently added organic matter influences the nitrification rate in soils, factors effecting crop growth may directly or indirectly influence nitrification. Legumes do not grow as well on acid soils as on neutral or slightly alkaline soils. Nitrification is influenced by the nitrogen content of the crop residue. In addition to the direct effect the hydrogen ion concentration may have upon nitrifying bacteria, an indirect effect may result too by retarding growth of legumes.

Plant growth usually is less on soils testing very low in available phosphorus or potassium than on soils higher in fertility. Nitrification may also be lower on soils very low in available phosphorus and potassium because of less plant residue or a nutrient deficiency for the microflora may exist. Studies were undertaken to determine if a relationship exists between pH, the amount of available phosphorus and potassium, and nitrification.

Historical

Several workers have shown that nitrification will take place in acid soils but as acidity increases the rate usually decreases. Fraps (28) found acid soils vary from no nitrification to a high nitrification. On samples taken from the Agronomy Farm at Ames, Iowa, Humfeld and Erdman (53) showed nitrification goes on until a pH of 4.4 to 4.8 is reached but from then on it proceeds very slowly. In pot studies conducted in a greenhouse using a Carrington loam soil that was loose and friable but with a rather high lime requirement, Stephenson (108) obtained fairly good nitrification. The nature of the compound to be nitrified plays an important role in acid soils according to Fred and Graul (34). Organic nitrogen nitrifies much more rapidly in acid soils than ammonium sulphate. In non-acid soils the reverse was true regardless of the source of nitrifying bacteria.

The hydrogen ion concentration increases in soil with nitrification. At the end of an eight-week incubation period, Allison and Sterling (3) found the decrease in pH amounted to 0.3 to 0.5 units where organic matter was the only nitrogen source. The addition of ammonium sulphate caused the pH to drop 1.6 to 1.9 units.

The addition of calcium carbonate or calcium oxide has been shown to stimulate nitrification in soils by many workers (3, 25, 26, 33, 48, 53, 88, 108, 116). Walker and Brown (116) added limestone of different amounts and degrees of fineness to a Grundy silt loam soil in southern Iowa. Samples were taken periodically during the subsequent five-year period for nitrification rate studies. The change in nitrifying power appeared to be associated with change in hydrogen ion concentration, which was a function of the degree of fineness and quantity of lime applied. Where limestone was applied in amounts beyond the lime requirements of the soil, the increase in nitrifying power induced per unit of limestone was reduced. Robinson and Bullis (99) obtained similar results. Soils responding to lime treatment gave a larger increase in nitrification than soils where no response was obtained from liming. On the soils from Manden, North Dakota, which Allison and Sterling (3) studied, lime caused a marked increase in nitrification even though all soils were above pH 6.1. Greatest effect was on the soils in the pH range 6.1 to 6.4.

Dean and Smith (25) found calcium limestone caused slightly more nitrification than dolomitic limestone when added to a Tama silt loam soil of pH 5.3. They attributed this to a slightly finer grind of the calcium stone. Calcium carbonate greatly increased nitrification on a Floyd soil with a pH of 5.0 according to Halvorson and Caldwell (48). The addition of gypsum on the same soil had little or no effect. This indicated that the hydrogen ion concentration limited the nitrification rather than a lack of calcium.

An alkaline reaction may limit nitrification as well as an acid reaction. A threshold pH value of 7.7 ± 0.1 was found by Caster, Martin and Buehrer (19) for the nitrification of ammonia type fertilizer above which the complete oxidation of ammonia will not occur and to which the pH values of soils must first be reduced before nitrification can proceed to completion. Halvorson and Caldwell (48) compared nitrification rates of two highly calcareous Webster soils with two non-calcareous soils taken in adjacent areas. A higher nitrification rate was found on the non-calcareous soils. The presence of large amounts of excess free calcium carbonate seemed to inhibit nitrification.

Brown and Hitchcock (17) showed nitrification was stimulated by the addition of small amounts of sodium chloride, sodium sulphate, or magnesium sulphate but they soon became toxic at higher concentrations. The toxic point for sodium chloride was found to be 0.02 per cent and for sodium sulphate

2.00 per cent. Calcium carbonate stimulated nitrification in alkali soils.

Conflicting results have been obtained in nitrification studies where phosphorus and potassium have been added. On five soils which Robinson and Bullis (99) studied, they found monocalcium phosphate had a beneficial effect on one, a depressing effect on three, and little effect on the other. Fraps and Sterges (30) added various phosphate materials to 18 low nitrifying Texas soils. Ammonium sulphate was added to all samples. Monocalcium phosphate alone increased nitrification on only four of the samples. Where used with calcium carbonate, nitrification was increased above the addition of calcium carbonate alone on 16 of the samples. The phosphate appeared to help in the oxidation of nitrite to nitrate.

In another study on nitrification in Texas soils, Fraps (28) found the addition of phosphate or potash increased nitrification in several soils as shown in the following table:

	Nitrification Rate (ppm)	
Active phosphoric acid (ppm)	0.02-0.04% N	0.04-0.06% N
0 - 10	21.4	49.7
10 - 20	26.3	42.8
20 - 30	25.9	43.0
30	28.9	49.0
Active potash (ppm)		
0 - 50	32.2	30.0
50 - 100	15.3	35.8
100 - 150	23.8	36.2
150	29.3	48.2

On the higher nitrogen soils potash increased nitrification but phosphorus did not. The reverse was true on the lower nitrogen soils. Fraps concluded in his report that phosphate is more effective than potash in stimulating nitrification.

Brown and Gowda (16) found the addition of either rock phosphate or superphosphate increased the nitrifying power of the soil. Dean and Smith (25) found rock phosphate applied to an acid Tama soil had no effect upon nitrification. The addition of 400 pounds per acre of 0-20-20 fertilizer on an acid Floyd soil did not increase nitrification materially according to Halvorson and Caldwell (48). As a result of his studies to reduce nitrate accumulation in fields near Rocky Ford, Colorado, Jensen (58) concluded the addition of superphosphate decreased the nitrifying activity.

Mack and Haley (81) found application of potassium compounds had no influence on some soils but increased nitrification of ammonium sulphate in others. Smith and Dean (106) applied 100, 200 and 500 pounds of potassium chloride per acre on calcareous soils in Iowa where corn responded to such applications. Nitrification was stimulated at first by the potassium chloride but apparently after about four weeks' time it was depressed.

Experimental

During 1950 the soil testing laboratory of Iowa State College received approximately 35,000 samples of soil from farmers' fields for testing. These samples were taken from all parts of the state and represent many soil types and management practices. After arrival at the laboratory, the samples were crushed to pass a 10-mesh screen, thoroughly mixed and stored in paper bags in trays. From these samples about 500 were selected for nitrification studies.

The samples were divided into two pH ranges, three ranges of available phosphorus, and two ranges of available potassium as follows:

	Group 1	Group 2	Group 3
pH	6.5 to 7.0	7.1 or above	---
Phosphorus (lbs./acre)	< 2.5	3 - 7.5	> 8.0
Potassium (lbs./acre)	< 150	> 150	---

pH was determined with Beckman pH meters using a water-soil ratio of 2:1.

Phosphorus was extracted from the soil with 0.03 N ammonium fluoride plus 0.025 N hydrochloric acid. The blue color developed by addition of ammonium molybdate and stannous chloride was measured with a Cenco photelometer.

Potassium* was extracted from the soil with 1 N neutral ammonium acetate and measured on a Perkin-Elmer flame photometer using the internal standard procedure with 100 ppm lithium nitrate.

The number of samples in each group was variable and in a few groups such as pH $>$7.1, P $>$8.0, and K $<$150 there were only a very few samples.

Questionnaires were sent to the county extension directors in the 67 counties from which the samples were taken relative to past management practices. Samples that had been taken from areas other than farmers' fields, such as gardens or orchards, were discarded. Peat or muck soils, those low in organic matter, or quite sandy soils were discarded also. Samples used in the test were medium to high in organic matter and loam to silty clay loams in texture. All samples were taken from 0 to 6 inches in depth. The samples had been in the storage racks over four months and were completely air dried.

For incubation studies, 10 grams of soil were placed in a 30 ml wide mouthed bottle and 2.5 ml of distilled water

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*Potassium determined by this procedure frequently is referred to as available or exchangeable potassium. Perhaps there are valid arguments against the use of either term, but in the absence of better terminology both are used in this text.

added by means of a fine glass nozzle. After one hole rubber stoppers were placed in the bottles, the samples were incubated three weeks at 35° C in a nearly saturated atmosphere. The nitrate was determined by the phenoldisulfonic acid method as previously described.

Soils in group 1 had a pH range of 6.5 to 7.0, a phosphorus content less than 2.5 pounds per acre, and potassium less than 150 pounds per acre. Soils in group 10 had the same pH range but phosphorus was above 8.0 pounds and potassium above 150 pounds per acre. Ten samples were selected from group 1 in which the nitrification rate was about 30 ppm and 10 samples were selected from group 10 in which the nitrification rate was about 50 to 60 ppm. To each of these samples 2.5 ml of solution containing 0.5 grams potassium dihydrogen phosphate per liter were added. This is equivalent to 130 pounds P_2O_5 per acre six inches of soil. The samples were stoppered and incubated as before.

Results

The average nitrification rates for soils separated into 12 groups according to two ranges of pH, three ranges of phosphorus, and two ranges of potassium are shown in Table 30. With the exception of groups 9, 11 and 12 there were 10 or

Table 30. Nitrification rates of soils classed into two pH ranges, three phosphorus ranges and two potassium ranges

Group	1	2	3	4	5	6	7	8	9	10	11	12
pH	6.5-7.0	6.5-7.0	>7.1	>7.1	6.5-7.0	6.5-7.0	>7.1	>7.1	6.5-7.0	6.5-7.0	>7.1	>7.1
P (lbs./acre)	<2.5	<2.5	<2.5	<2.5	3-7.5	3-7.5	3-7.5	3-7.5	>8.0	>8.0	>8.0	>8.0
K (lbs./acre)	<150	>150	<150	>150	<150	>150	<150	>150	<150	>150	<150	>150
Corn												
Number samples	16	13	10	12	16	12	21	19	18	20	4	20
Nitrification rate (ppm)	40.8	37.4	56.0	46.2	44.3	43.6	53.7	54.9	42.5	47.5	53.0	58.1
Oats, with or without seeding												
Number samples	10	10	13	10	13	15	16	11	7	10	2	6
Nitrification rate (ppm)	32.1	38.6	49.9	50.0	45.4	48.1	54.4	56.5	51.3	49.4	38.1	57.7
All crops												
Number samples	47	42	46	45	48	47	47	47	37	36	14	37
Nitrification rate (ppm)	37.2	39.4	52.0	45.9	44.2	45.9	54.3	55.7	44.7	48.8	53.2	58.7
K >150 over <150 ppm		+2.2		-6.1		+1.7		+1.4		+4.1		+5.5
P 3-7 over <2.5 ppm					+7.0	+6.5	+2.3	+9.8				
P >8 over 3-7 ppm									+0.5	+3.1	-1.1	+3.0
P >8 over <2.5 ppm									+7.5	9.4	+1.2	+12.8
pH >7.1 over 6.5-7.0 ppm			+12.2	+6.5			+10.1	+9.8			+8.5	+9.9

more samples in each group in which the soils were taken from either corn fields or fields planted to oats with or without seedings. There was no consistent difference between samples taken for either corn or oats. There was a much greater variation between samples within each group than between crops.

More than 35 samples were tested in all groups except number 11 which had only 14. Seldom do you find calcareous soils in Iowa that are high in available phosphorus and low in available potassium. The difference between groups 3 and 4 may be partially accounted for by the areas from which the samples were taken. Almost all soils that are calcareous and contain less than 150 pounds per acre of exchangeable potassium are from the Clarion-Webster soil association area. These soils are relatively high in organic matter. Approximately one-third of the soils in group 4 which are calcareous but high in exchangeable potassium are from the Ida soils in western Iowa. These soils are much lower in organic matter than the Clarion-Webster soils. Although soils low in organic matter were discarded for this test, the difference in organic matter is probably responsible for the difference in nitrification rates.

Soils that contain more than 150 pounds per acre of exchangeable potassium had a slightly higher nitrification rate than soils containing less than 150 pounds. The largest difference in nitrification rate between soils high and low in potassium occurred on soils high in available phosphorus.

A definite relationship was found between nitrification rate and available phosphorus (Table 30). Soils in groups with phosphorus range of 3 to 7.5 pounds per acre had a nitrification rate from 2.3 to 9.8 ppm greater than soils having less than 2.5 pounds available phosphorus per acre. In three of the four comparisons the difference was greater than 6 ppm. The mean rate of nitrification for soils having more than eight pounds available phosphorus per acre was only slightly higher than the mean rate in the 3.0 to 7.5 pound range. The nitrification rate was definitely higher in the groups containing more than eight pounds per acre available phosphorus compared with the groups containing less than 2.5 pounds. In this comparison, the mean nitrification in three of the four high phosphorus groups was more than 7.0 ppm above the low phosphorus groups. The one low group was number 11 which had only a few samples.

Although pH above 6.5 is considered optimum for nitrification, in all six comparisons in Table 30, soils with a pH above 7.1 had a nitrification rate considerably higher than soils in the pH range of 6.5 to 7.0. The presence of a little excess calcium carbonate apparently stimulated the nitrification process.

The addition of potassium dihydrogen phosphate to soils at the time of incubation greatly increased the nitrification rates regardless of the initial phosphorus or potassium levels (Table 31). There was considerable variation in the increase among the 10 samples in each group and the difference between group means is not significant.

Discussion

Several workers have shown the addition of calcium carbonate increased nitrification of soils, especially if ammonium sulphate was the source of ammonia. Allison and Sterling (3) found addition of lime to soils in North Dakota with a pH above 6.3 increased nitrification. The presence of naturally occurring calcium carbonate stimulates nitrification also, according to the results reported herein. This does not agree, however, with the work reported by Halvorson and Caldwell (48). Not many calcareous soils in Iowa have a pH above 7.7 which was reported by Caster, Martin and Buehrer (19) as the upper threshold pH for nitrification of ammonia.

The availability of phosphorus and potassium in soils may influence nitrification in several ways. First, there may be a direct effect upon the activity of the microorganisms responsible for nitrification. Second, plant growth is likely

Table 31. The influence of potassium dihydrogen phosphate upon nitrification rates of soils of two fertility levels

Sample number	Nitrification rate ppm H_2O	Nitrification rate ppm KH_2PO_4	Increase in nitrification ppm
	Group 1 - pH 6.5 - 7.0; P < 2.5; K < 150		
9222	32.1	42.9	10.8
9640	29.7	40.2	10.5
10707	31.1	42.7	11.6
10874	26.0	35.7	9.7
12536	28.2	49.8	21.6
12914	30.6	57.2	26.6
9790	38.1	45.7	7.6
10618	22.1	33.5	11.4
12279	24.9	37.0	12.1
12557	36.2	51.2	15.0
		Average increase = 13.7 ppm	
	Group 10 - pH 6.5 - 7.0; P > 8.0; K > 150		
10580	51.8	59.1	7.3
12895	55.6	82.0	26.4
10627	76.0	80.3	4.3
10821	51.6	64.4	12.8
11015	61.6	72.6	11.0
11512	58.6	82.6	24.0
11649	50.6	60.6	10.0
11905	55.0	75.4	20.4
12377	51.8	69.6	17.8
11580	83.0	109.0	26.0
		Average increase = 16.0 ppm	

to be less on soils low in available phosphorus and potassium in comparison to soils high in these elements. This will result in differences in the quantity and composition of plant residues. Third, the microorganisms causing the initial decomposition of the carbohydrates and proteins in crop residues may be influenced by the levels of phosphorus and potassium.

The experiment reported in Table 31 on the influence of potassium dihydrogen phosphate on nitrate production was undertaken to ascertain whether or not the greater nitrification in soils high in available phosphorus and potassium could be explained directly by the high nutrient levels. The stimulation in the soils high in phosphorus and potassium is difficult to explain. It may be due to stimulation of different microfloras by alterations in availability of organic nitrogen in the soil.

CORRELATION OF NITRIFICATION RATES WITH RESPONSE TO NITROGEN FERTILIZER APPLICATION

Introduction

For many years in the Midwest, emphasis has been placed upon supplying the nitrogen requirements of corn through growth of legumes and application of manure. The use of commercial nitrogen fertilizers on corn is still a relatively new practice in Iowa. Most of the field research on use of nitrogen fertilizers in Iowa has been conducted since 1943 (86, 87). As previously indicated, several workers have been able to show a correlation between nitrification rates and corn yields on various rotation plots which reflect the influence of several years' growth of legumes or many applications of manure. No published reports were found, however, showing the relationship of nitrification rates with response to nitrogen fertilizer applications to corn.

Experimental

The nitrogen fertilizer experiments on corn that were selected for correlation with nitrification rate were located on fields where the farmers were following cultural, soil management and cropping practices typical of their area. Soil samples for the nitrification studies were taken from the sites of the individual field experiments for the years 1943 through 1950 with the exception of 1947. Adverse climatic conditions caused the results of the fertilizer experiments to be very erratic in the latter season and they were not included in the correlation tests. All of the soil samples were taken to a depth of six inches before the application of fertilizer to the area. Each sample is a composite of a large number of borings with a soil auger. After air drying, the samples were thoroughly mixed and stored in either pint or quart fruit jars.

The design of the field experiments varied considerably over the period of 1943 through 1950, but all of the experiments were replicated several times. On fields where rates or methods of nitrogen application were studied, the treatments were replicated four to nine times in randomized designs. In most instances these experiments received a blanket application of 200 pounds per acre of 0-20-10 fertilizer. On many of the fields factorial experiments of three to four replications each were conducted in which nitrogen, phosphorus and potassium were applied singly and in all possible combinations. The yield increases from nitrogen fertilizer used in correlation with nitrate production were obtained by subtracting the yields of all of the nitrogen treatments from the yields of all the no-nitrogen treatments.

For correlation with nitrification data, yield increases from 40 or 60 pounds application of nitrogen fertilizers to corn were used. No distinction was made to various methods of application which included banding in the plow-sole, broadcasting and plowing under, early side-dressing when the corn was four to ten inches high and late side-dressing when the corn was 24 to 30 inches tall. Neither was a distinction made relative to the source of inorganic nitrogen fertilizers used in the experiments.

Nitrification studies were conducted using 25 grams of soil that had been crushed to pass a 10-mesh screen. The samples were placed in pint milk bottles and 6.25 ml of distilled water added with a fine glass nozzle to assure uniform distribution. The bottles were covered with one hole rubber stoppers and incubated at 35° C for a period of three weeks in a nearly saturated atmosphere.

Nitrate nitrogen was determined by the modified phenoldisulfonic acid procedure that was previously described.

Results

The response obtained from application of nitrogen fertilizers on corn is greatly influenced by the thickness of stand.

A good response from application of nitrogen fertilizer may be obtained with a relatively thick stand of corn where no response would be obtained on the same soil with a thin stand. The results from the field experiments were separated into the following three groups: less than 8500 stalks per acre referred to as a thin stand, from 8500 to 10,500 stalks per acre as a medium stand, and more than 10,500 stalks per acre as a thick stand.

The yield of corn, the increase in yield of corn from application of nitrogen together with the initial nitrate in the soil samples and the nitrification rates are given in Tables 32, 33, and 34. A great variability in yields of corn and response to nitrogen is found in all three groups. The amount of nitrate present and the nitrification rates vary considerably too as would be expected. The mean yield of corn for both the thin and thick stands was about 60 bushels per acre, but the average response from application of nitrogen fertilizers was 14.2 bushels per acre on the thick stands and 5.4 bushels on the thin stands. These relatively greater responses to nitrogen were obtained on the thick stands notwithstanding the fact that the general fertility level may also have been higher. The average nitrification rate on the thick stands was 50.9 ppm compared to 39.2 ppm on the thin stands.

Table 32. The relation of corn yield responses to nitrogen fertilization and soil nitrification rates on fields with less than 8500 stalks per acre

Sample number	Year	Stalks per acre	Corn - bu./acre Non-treated	Corn - bu./acre Increase from 40 or 60 lbs. N	Original nitrate ppm	Nitrification rate ppm
1809	1943	8000	57.2	7.8	5.0	49.2
1811	1943	5000	46.0	4.8	5.4	42.2
1813	1943	7590	80.7	5.0	3.6	57.0
1814	1943	7040	48.2	7.9	13.8	51.7
1815	1943	8080	55.4	7.2	12.4	28.4
1816	1943	7630	61.0	-1.3	4.0	45.7
1934	1944	7000	58.3	14.0	4.2	16.9
1939	1944	7000	49.8	9.2	3.2	7.8
1944	1944	7000	39.1	17.6	1.6	15.0
1958	1944	7500	52.3	8.6	4.2	27.0
1985	1944	7500	60.5	9.8	3.8	5.8
1991	1944	7000	80.7	2.4	10.1	41.3
1994	1944	8000	81.0	1.4	7.3	44.7
2018	1944	7500	29.0	-4.6	14.6	50.6
2024	1944	7100	55.7	7.5	6.8	51.0
2586	1945	7800	74.8	3.2	7.5	23.9
2590	1945	7500	54.2	0.6	9.8	74.2
2595	1945	7000	75.0	0.0	2.5	51.5
3041	1946	8000	80.3	0.6	8.6	50.4
F491	1949	8020	62.6	7.3	3.3	51.1
Mean			60.0	5.4		39.2

Table 33. The relation of corn yield responses to nitrogen fertilization and soil nitrification rates on fields with 8500 to 10,500 stalks per acre

Sample number	Year	Stalks per acre	Corn - bu./acre		Original nitrate ppm	Nitrification rate ppm
			Non-treated	Increase from 40 or 60 lbs. N		
692	1943	9850	65.2	24.3	7.4	19.1
1812	1943	8570	45.9	18.9	8.4	36.6
1928	1944	8600	41.8	7.4	14.8	55.9
1949	1944	10,000	73.9	23.0	2.6	25.2
1961	1944	10,000	89.0	10.3	4.8	10.4
1964	1944	10,000	66.4	16.1	3.6	48.3
1982	1944	9300	77.3	5.0	21.2	62.0
1997	1944	9500	91.2	0.0	6.3	50.5
2003	1944	9200	31.0	18.8	3.6	14.4
2012	1944	8800	72.8	12.0	4.8	53.6
2135	1944	9200	94.5	10.6	2.8	53.4
2587	1945	10,000	88.0	3.9	11.0	74.0
2678	1946	10,500	90.7	2.0	6.3	58.7
2679	1946	8800	71.2	11.0	3.5	20.3
2682	1946	9000	71.2	13.0	3.3	44.7
F370	1948	10,160	90.7	4.8	4.1	67.1
F828	1950	9450	31.6	5.4	6.4	59.4
F960	1950	8840	60.2	12.0	33.1	76.9
F961	1950	9800	43.5	14.3	28.5	53.5
F962	1950	9950	79.5	9.5	13.7	73.3
F1013	1950	9900	76.0	10.2	14.0	60.8
F1014	1950	9500	61.6	18.5	11.8	83.0
F965	1950	10,500	81.8	6.2	21.4	61.6
F819	1950	10,000	67.0	0.1	20.8	56.8
Mean			69.3	10.7		50.8

Table 34. The relation of corn yield responses to nitrogen fertilization and soil nitrification rates on fields with more than 10,500 stalks per acre

Sample number	Year	Stalks per acre	Corn - bu./acre Non-treated	Corn - bu./acre Increase from 40 or 60 lbs. N	Original nitrate ppm	Nitrification rate ppm
1967	1944	11,000	36.2	13.1	2.4	6.5
2009	1944	12,000	52.1	17.6	3.2	42.8
2133	1944	13,000	111.5	12.5	2.8	11.4
F371	1948	11,760	104.4	2.8	4.0	87.6
F373	1948	13,860	89.3	16.2	3.3	40.1
F376	1948	13,910	98.4	4.9	7.8	53.2
F378	1948	12,310	92.8	4.5	4.3	60.5
F379	1948	16,770	104.7	7.9	2.4	58.6
F407	1948	13,030	81.0	6.8	4.0	52.8
F489	1949	13,850	36.5	7.1	2.6	48.6
F490	1949	13,650	46.2	7.2	3.0	65.0
F491	1949	12,880	62.6	22.4	3.3	51.1
F492	1949	11,310	26.5	25.6	2.8	66.3
F493	1949	14,060	61.6	33.2	2.6	66.5
F494	1949	13,850	32.2	39.5	3.5	70.5
F806	1950	14,540	63.7	3.6	7.5	59.7
F808	1950	12,010	40.3	7.2	8.9	64.9
F809	1950	14,130	72.9	1.8	26.3	76.5
F811	1950	10,820	48.5	5.7	7.3	57.7
F820	1950	10,910	52.8	16.2	4.9	55.1
F824	1950	10,620	43.3	19.8	8.8	46.6
F827	1950	16,330	46.1	29.1	5.1	51.9
F955	1950	11,540	21.5	13.5	5.1	48.4
F956	1950	10,790	34.2	30.0	14.7	26.1
F957	1950	13,170	35.2	24.0	8.8	25.8
F958	1950	12,020	38.5	19.5	14.4	33.4
F959	1950	10,980	71.3	-1.2	18.2	58.4
F963	1950	11,760	76.6	10.2	17.0	65.0
F966	1950	11,880	23.2	10.6	4.8	24.8
Mean			58.8	14.2		50.9

On the medium stand of corn, the average yield was 69.3 bushels per acre which was about 10 bushels higher than either of the other groups. The response of corn to nitrogen fertilizer was 10.7 bushels per acre or about half way between the thin and thick stands. The average nitrification rate was the same as for the thick stand of corn.

An increase of more than 10 bushels per acre from application of nitrogen fertilizer was obtained on 58 per cent of the fields with medium and thick stands. In contrast to this only 10 per cent of the fields with thin stands had an increase above 10 bushels per acre.

In Figures 10, 11 and 12 are plotted the increase in yield of corn from application of nitrogen against nitrification rates. The relationship between response to nitrogen and nitrification rate on fields with thin stands is expressed by the regression equation, $y = -.185x + 12.72$ where y represents the yield response and x represents the gain in nitrate nitrogen during three weeks' incubation. The regression coefficient (r) was found to be $= -.630^{**}$ which is significant at the one per cent level. In this group five out of seven fields with nitrification rate less than 40 ppm showed an increase in yield of more than nine bushels per acre from nitrogen application.

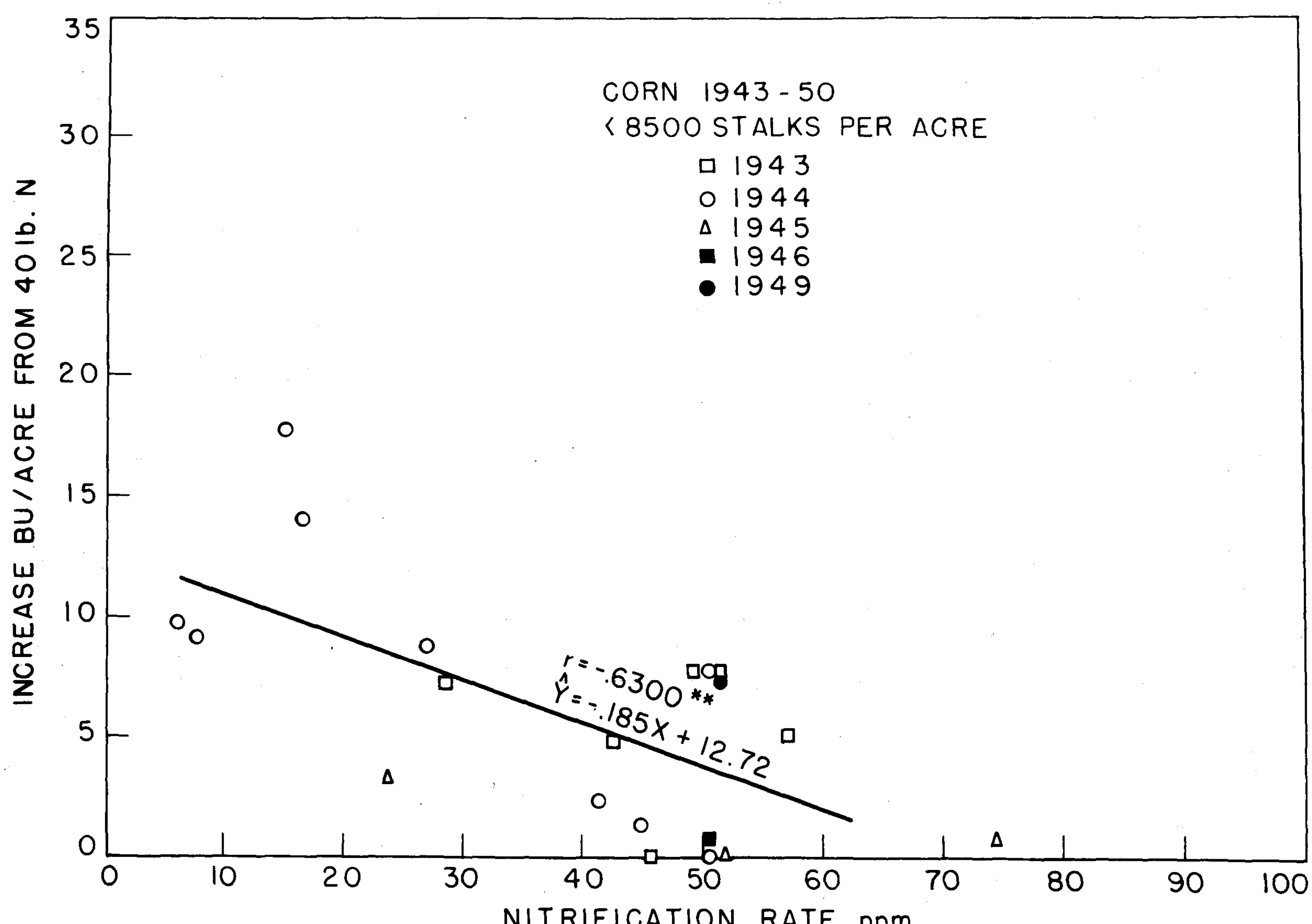

Figure 10. The relationship between nitrification rate and the increase from 40 pounds nitrogen in yield of corn having less than 8500 stalks per acre

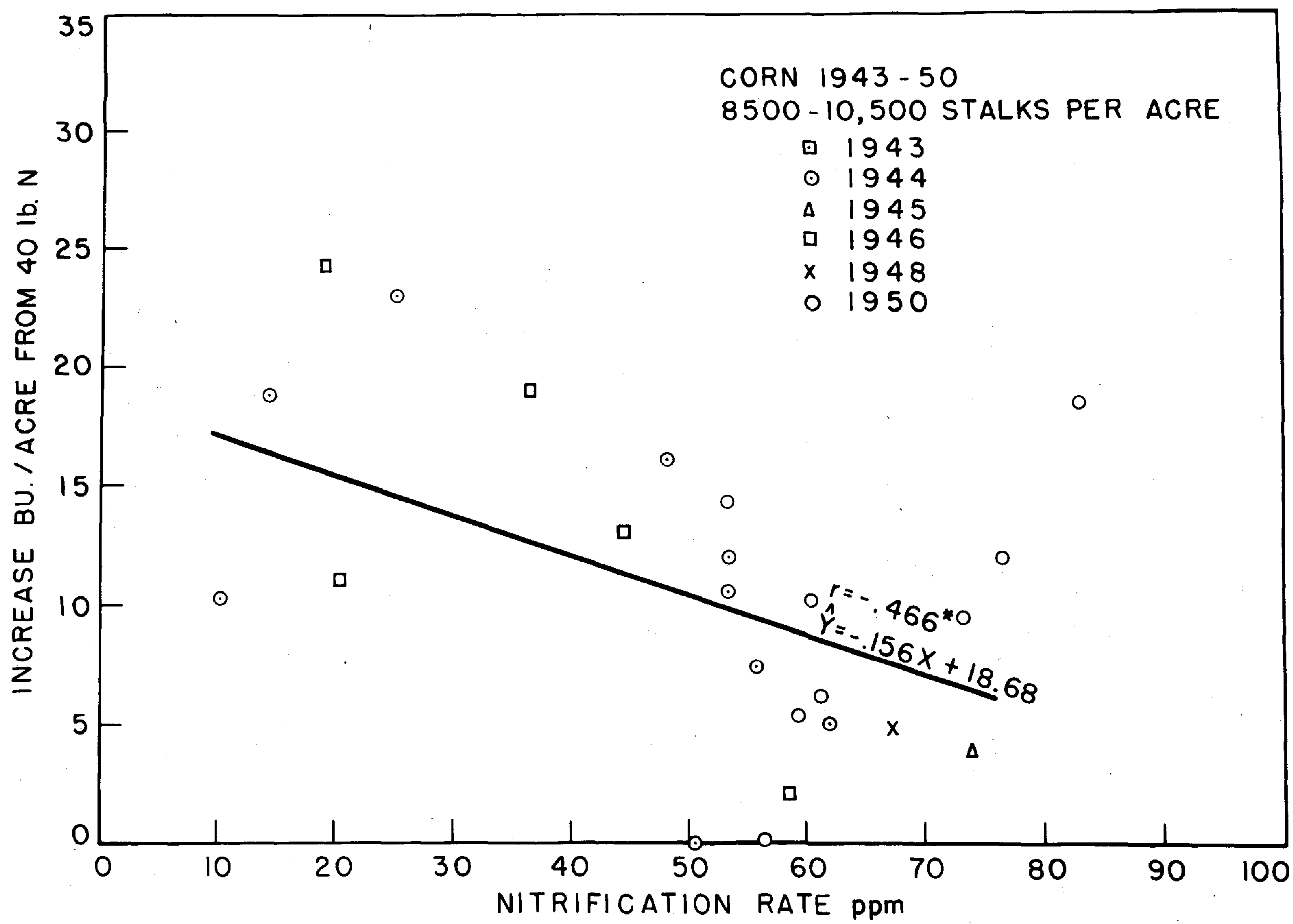

Figure 11. The relationship between nitrification rate and the increase from 40 pounds nitrogen in yield of corn having 8500 to 10,500 stalks per acre

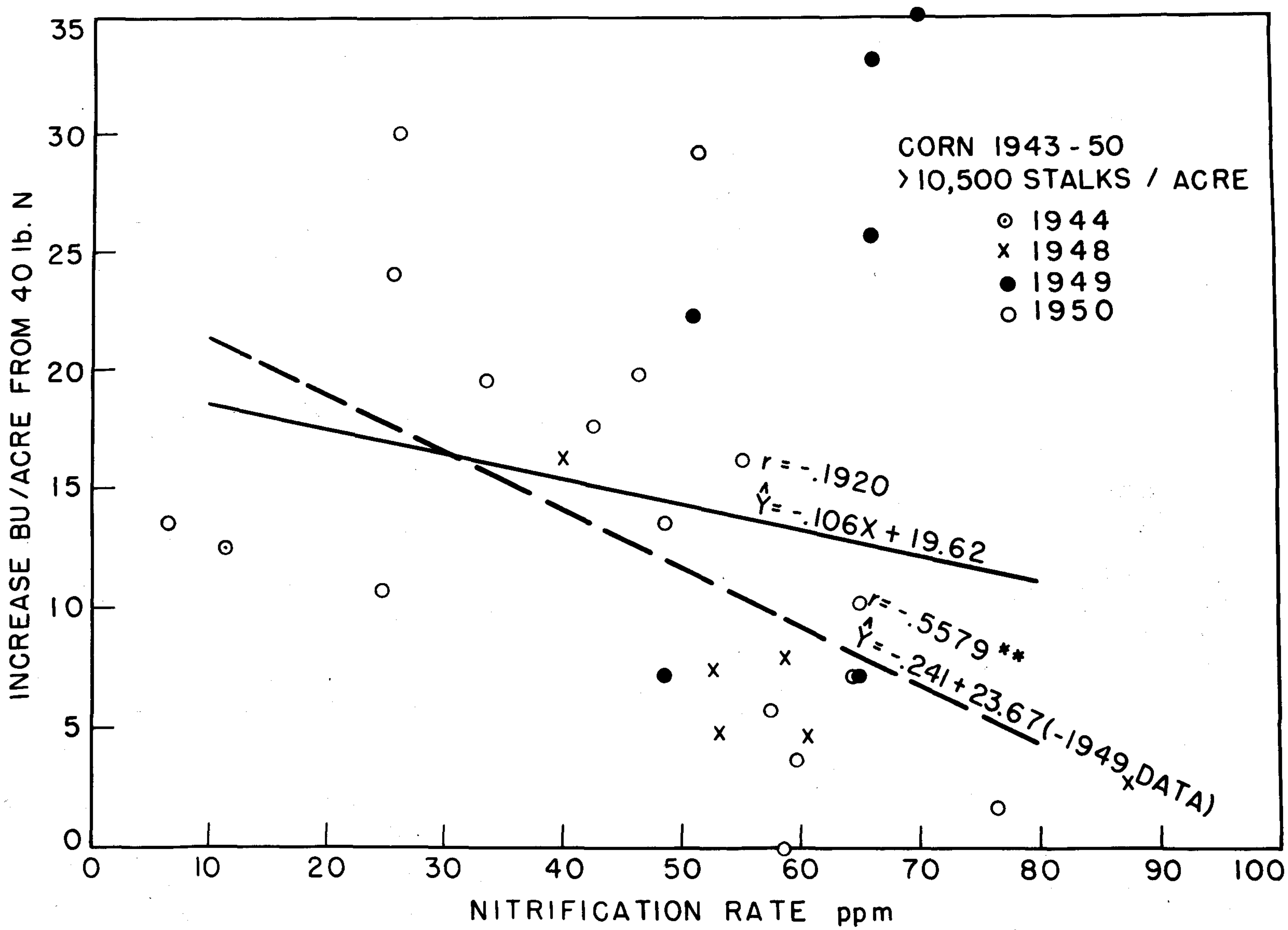

Figure 12. The relationship between nitrification rate and the increase from 40 pounds nitrogen in yield of corn having greater than 10,500 stalks per acre

The regression equation expressing the relationship between yield increase and nitrification rate for the medium stands was found to be $y = -.156x + 18.68$. The regression coefficient (r) is -.466* which is significant at the 5 per cent level. In this group all fields with a nitrification rate less than 50 ppm showed an increase of more than 10 bushels per acre from application of nitrogen.

The relation of yield response to nitrification rate was more variable among fields with heavier stands. Moreover, the influence of seasons appears to be more pronounced with thick than thin stands. Where the data from all years are considered the regression equation was found to be $y = -.106x + 19.62$ and the regression coefficient (r) = -.1920 which is not significant. The 1949 season was optimum for corn production and large increases were obtained on fields which were relatively fertile and above average in production. If the data for 1949 are omitted then the regression equation becomes $y = -.241 + 23.67$ and the regression coefficient (r) = -.558** which is significant at the one per cent level. In all instances where the nitrification rate was less than about 50 ppm, the yield increase was more than 10 bushels per acre from application of nitrogen fertilizer.

Discussion

The response from application of nitrogen fertilizers on corn is markedly influenced by management practices and climatic conditions. The period of greatest nitrogen requirement by corn occurs in early summer during the tasseling period. Several workers have found that greatest nitrification takes place in June under field conditions. The amount of nitrogen that accumulates in the soil previous to the period of greatest need by corn will be greatly influenced by management and cultural practices as well as climatic conditions. Heavy rains during the last of June may leach the soil of nitrate nitrogen and the corn may have to rely upon the decomposition of more organic nitrogen in order to obtain its requirements. In contrast to this, climatic conditions may be such that nitrate accumulates in the soils and although the nitrification rate may not be high, the corn may obtain a sufficient quantity when needed most. Thickness of stand will greatly influence whether or not the nitrogen supply is adequate.

Although there was considerable seasonal variability, the data do show that nitrification rate as determined by incubation of soil samples gave results that were definitely correlated with response to application of nitrogen fertilizers on corn when thickness of stand is considered. A closer grouping of points about a regression line should not be expected when no separation is made relative to soil type, method of application of nitrogen fertilizer, variations in climatic conditions from one year to another, and when each point represents an individual field.

In studies conducted in Iowa since 1943, no consistent yield response differences were found among methods of nitrogen fertilizer application. Under certain conditions, one method might give a little better results than another but under other conditions the results might be reversed. The differences were greatly influenced by climatic conditions and cultural practices. Even though the method of application may have influenced the results somewhat, it did not seem advisable to attempt separation of fields on this basis. The same was true of sources of inorganic nitrogen fertilizers.

The regression equations relating response to application of nitrogen fertilizer on corn to nitrification rates are calculated using the yield increase as the independent variable. Yield increases are subject to many of the same errors of measurement that influence nitrification rates. The real value of the regression equations from the standpoint of soil testing is how well will they predict what will happen on other fields in the ensuing years. Higher correlation values could be obtained if the regression equations were determined separately for each year's data, assuming there were sufficient experiments conducted during each year. However, in using nitrification rates as a tool for predicting nitrogen response, the results for several years must be considered. Unfortunately, the thickness of stands were not uniformly distributed over the period of years. The thin stands predominately occurred in the earlier years of field studies. In more recent years, fields with better stands were selected for the experiments. This gives a certain bias to the results.

Perhaps the most significant fact revealed by the data is that in the seven years of results reported, whenever the nitrification rate was less than 40 ppm regardless of stand, a profitable increase was obtained from application of 40 pounds per acre of nitrogen fertilizer. Where the stands were thicker than 8,500 stalks per acre, the increase was above 10 bushels per acre from application of nitrogen fertilizers on fields having a nitrification rate less than 50 ppm. More than half of the fields with a nitrification rate above 50 ppm had an increase less than eight bushels per acre from application of nitrogen fertilizer. In all groups, however, profitable responses were obtained from nitrogen fertilizer on some fields with nitrification rates above 50 ppm. The percentage of such fields increased with increase in thickness of stand. With good cultural practices, freedom from disease and insects, good varieties and stands and optimum climatic conditions, increases from nitrogen fertilizer application can be obtained on fields yielding over 100 bushels per acre without nitrogen fertilizer. Obviously, the fertility level of such fields must be high and nitrification rates will be correspondingly high.

The data presented in general are similar to correlation of soil tests to crop response from application of other nutrient elements such as phosphorus or potassium. At the low or extremely high nutrient levels as indicated by soil tests, the response can be predicted fairly accurately. In the medium range, however, factors other than fertility will greatly influence the results obtained from application of fertilizer containing that element.

GENERAL DISCUSSION AND SUMMARY

Nitrification rate has been proposed as a method for evaluating nitrogen requirement of soils. Studies were conducted to find procedures which would control some important factors influencing results and which would speed up analysis without sacrificing accuracy.

Loss of moisture during two- or three-week incubation periods from soil samples in pint milk bottles covered with several folds of cheesecloth was found to be quite large. This loss was eliminated when one hole rubber stoppers were used in place of the cheesecloth. Aeration through the one hole stopper was adequate for nitrification.

Nitrification takes place over a fairly wide range of soil moisture. When water was mixed with the soil, the optimum moisture was about 25 per cent for soils medium to high in organic matter and loam to silty clay loam in texture. On soils in which excess water was removed by pressure or tension, greatest nitrification took place at 100 cms water tension. This was equivalent to 25 to 35 per cent moisture in the soils studied.

No difference in nitrate production was obtained in incubating 25 or 100 gram samples in milk bottles. In nitrification studies with 10 grams of soil in 30 ml bottles, the results were consistently lower by about 5 ppm than those from 25 or 100 gram samples in milk bottles.

The type of container had no influence on nitrification rates when one hole rubber stoppers were used to cover them.

A temperature of 55° C for three days apparently destroyed the activity of nitrifying organisms since they were unable to recover when incubated for another 18 days at 35° C.

Aggregate size apparently had little influence upon nitrate production. Samples mixed by hand to eliminate clods larger than three-eighths of an inch in diameter had about the same nitrification rate as samples crushed to pass 10 or 20 mesh screens.

Several modifications for increasing speed and ease of analysis were made in the phenoldisulfonic acid procedure for nitrate determination. Specially constructed trays permitted handling 12 samples as a unit. Measured quantities of solutions were added to obtain a given volume rather than bringing the solutions to volume in a volumetric flask. Measurement of the intensity of the yellow color produced was made on a photometer with a calibration curve instead of direct comparison with standard solutions.

A composite sample of 10 cores taken with a soil tube to a depth of six inches was found to be representative of the soil conditions on first- and second-year corn plots at Ames and Clarinda for all three sampling dates. The variability in nitrification among composite samples was greater than between duplicate determinations but was much less than among replicated plots.

A seasonal difference was found in both nitrate production and initial nitrate content of soils. The difference, however, was less than 10 ppm on almost all of the plots.

The nitrification rate of soil samples from first-year corn following meadow was markedly higher than from second-year corn plots. The nitrate production following clover was higher on first- than on second-year corn, but the difference was not as large as found on the meadow plots. Corn following meadow was higher in yield than corn following clover. Nitrification rates paralleled the yields in the two treatments.

A relationship was found between nitrification rate and the levels of available phosphorus and potassium among a series of soil samples submitted for testing. Nitrate production was less on soils low in these elements than on soils medium to high. A relation was also found between nitrification rate and soil reaction. During the incubation period about 10 ppm more nitrate was produced in soils with pH above 7.1 compared to soils in pH range 6.5 to 7.0.

A significant correlation was obtained between nitrification rates and response of corn to the application of nitrogen fertilizers in field experiments conducted during the years 1943 through 1946 and 1948 through 1950. On all fields where the nitrate production was less than 40 ppm, a profitable increase in yield of corn was obtained from application of nitrogen fertilizers.

Variations in climatic conditions from one year to another greatly influenced the relationship between nitrate production and response to application of nitrogen fertilizers. Some of the differences might be accounted for by the variability in thickness of stands during different seasons.

Nitrification rate procedures including incubation of soil samples and analysis of nitrate by phenoldisulfonic acid method have been modified to permit rapid analysis as required for use in a soil testing laboratory. Corn growing on soils in Iowa which fail to produce more than 50 ppm nitrate in a three-week incubation period are very likely to respond profitably from application of 40 pounds nitrogen per acre. Increases in yield from nitrogen fertilization will be obtained at higher nitrification rates with thick stands than with thin stands.

Although a relationship was found to exist between nitrate production, pH, and available phosphorus and potassium, it has not been determined how to use this information in making nitrogen recommendations for corn based on nitrification rate. More research on field conditions is needed to determine the influence that addition of phosphate and potash fertilizers may have upon nitrogen availability on soils testing low in phosphorus or potassium.

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ACKNOWLEDGMENT

The writer wishes to express his appreciation to Dr. W. V. Bartholomew for the assistance given throughout the course of this study. To Mrs. Helen Heidel, the writer is especially grateful for the help given in conducting the research and in preparation of the dissertation. The soil samples used in correlating nitrification rate with response by corn to nitrogen fertilizer application were taken by Dr. L. B. Nelson, Dr. C. A. Black, Prof. Lloyd C. Dumenil and Dr. George Stanford, and to these men, the writer is indebted for the use of the samples. Prof. John Hanway was instrumental in obtaining some of the soil samples used in studying nitrification procedures and Dr. T. M. McCalla furnished samples of known nitrate content and nitrification rate production for use in comparison of techniques. For these courtesies the writer is appreciative.